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Treatment of Experimental Ileus by Hypertonic Saline Solutions.

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Largely due to the observations of Hughson and Scarff[1] that the intravenous administration of hypertonic sodium chloride solutions increased intestinal movement, hypertonic saline solutions have been used clinically in the treatment of ileus. To determine the relative effects of various hypertonic saline solutions the following investigation was undertaken. One hundred and twenty-four observations were made on 63 animals. Of this group only 113 are included in the present study, because in 8 of the instances a physiologic Ringer's solution was employed and in 3 the results obtained could not be used because of a leak in the intestinal balloon. In each instance, kymographic tracings were made of the intestinal movements, and the results presented are based on an analysis of these tracings. In 75 of the instances, the observations were made with the animal submerged in a normal saline bath[2] ("open abdomen" technic), whereas in 38 the observations were made in the intact, unanesthetized animal ("closed abdomen" technic), which was made possible by the use of a technic previously described.[3] The following

[1] Hughson, W., and Scarff, J. E., *Bull. Johns Hopkins Hosp.*, 1924, **35**, 197.

[2] Ochsner, Alton, Gage, I. M., and Cutting, R. A., *Arch. Surg.*, 1930, **20**, 802.

[3] Gage, I. M., Ochsner, Alton, and Cutting, R. A., PROC. SOC. EXP. BIOL. AND MED., 1931, **24**, 264.

solutions were used for intravenous injection: sodium chloride, 20%; "hypertonic" Ringer's solution; "hypertonic" Hartmann's combined solution; sodium chloride, 20%, and calcium chloride, 5%; sodium chloride, 20%, and calcium chloride, 0.5%; sodium chloride, 20%, and potassium chloride, 0.5%. Ten observations were made on normal animals, 13 on animals with 24-hour intestinal obstruction, 49 on animals with 48-hour obstruction, and 41 on animals with 72-hour obstruction.

Twenty-one observations were made following the intravenous injection of 20% sodium chloride solutions, employing the "open abdomen" technic. The average dose was 8.4 cc. per kilo of body weight. In 90.4% of the instances there was an increase in intestinal activity, in 4.7% there was no change, and in 4.7% there was a decrease in intestinal activity. The average increases in tone and amplitude were 28.6 and 5.05 mm., respectively. The average duration of this activity was 11.1 minutes. The percentage increase in blood chlorides was 23.1%. "Hypertonic" Ringer's solution (sodium chloride, 18%; calcium chloride, 0.52%; potassium chloride, 0.6%) was used in 67 instances. The "open abdomen" technic was used in 39 and the "closed abdomen" technic in 18. The average dose in the former group was 2.5 cc. per kilo of body weight. In 97.3% of these observations there was an increase in gut activity, whereas in 2.6% there was no change. The average increases in tone and amplitude were 61.2 mm. and 18.4 mm., respectively. The average duration of the increased activity was 17.5 minutes. The average percentage increase in blood chlorides was 22.5%. The average dose employed in the "closed abdomen" experiments, using "hypertonic" Ringer's solution was 1.17 cc. per kilo of body weight. In 94.7% there was an increase in gut activity, whereas in 5.5% there was no change. The average increases in tone and amplitude were 13.1 mm. and 2.9 mm., respectively. The average duration of the increased activity was 17.6 minutes. The average percentage increase in blood chlorides in the "closed abdomen" experiments was 23.4%.

Twenty observations were made concerning the effect of "hypertonic" Hartmann's solutions* (sodium chloride, 11.7%; sodium lactate, 5.6%; potassium chloride, 0.74%; calcium chloride, 0.54%, obtained by adding 5 cc. to the contents of a 20 cc. ampoule of concentrated Hartmann's solution). The average dose employed was 1.16 cc. per kilo of body weight. In 95% there was an increase in activity; in 5% no change. The average increases in tone and am-

* Hartmann's solution was kindly furnished us by the Eli Lilly Company.

plitude were 20 mm. and 3.6 mm., respectively. The average duration of this increased activity was 20.2 minutes.

Four observations were made concerning the effect of sodium chloride, 20%, and calcium chloride, 5%. The average dose employed was 2.7 cc. per kilo of body weight. There was an increase in gut activity in all observations. The average increases in tone and amplitude were 25.7 mm. and 12.5 mm. respectively. The average duration of increased activity was 17.2 minutes.

Five observations were made concerning the effect of sodium chloride, 20%, and calcium chloride, 0.5%. The average dose was 2.2 cc. per kilo of body weight. In all instances there was an increase in gut activity. The average increases in tone and amplitude were 73.8 mm. and 16.6 mm., respectively. The average duration of the increased activity was 19.2 minutes.

Six observations were made concerning the effect of sodium chloride, 20%, and potassium chloride, 0.5%. The average dose employed was 4.6 cc. per kilo of body weight. There was an increase in intestinal activity in 83.3%, in 16.6%, no change. The average increases in tone and amplitude were 58 mm. and 19.4 mm., respectively. The average duration of the activity was 19 minutes.

It is evident from these experiments that "hypertonic" Ringer's solution or "hypertonic" Hartmann's solution is more efficacious than is 20% sodium chloride in stimulating obstructed intestine. Even though much larger doses of sodium chloride were employed (8.4 cc. per kilo of body weight) there was an increased intestinal activity in only 90.4%, as compared with 97.3% in which "hypertonic" Ringer's solution was used. The increases in tone resulting from the intravenous administration of sodium chloride, 20%, and "hypertonic" Ringer's solution were 28.6 mm. and 61.2 mm., respectively. The increases in amplitude were 5.05 mm. and 18.4 mm., respectively. The duration of the increased activity was 11.1 minutes and 17.5 minutes, respectively.

Conclusions. Hypertonic sodium chloride and hypertonic salts containing potassium and calcium chloride are efficacious in stimulating normal and obstructed intestine. Those solutions which contain calcium and potassium are more efficacious than when sodium chloride alone is used. Apparently the combination of sodium chloride and calcium is more efficient in its action than the combination of sodium chloride and potassium chloride.

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Experimental Production of Gas Bacillus Infection with Wool Implantations.

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The organisms producing gas bacillus infection are widely distributed in nature, the most frequent source being from contaminated soil by the excreta of both man and animals. That the organisms can, and do occur in finished products made from both wool and hair from animals were reported by the author.[1] It was demonstrated at that time both clinically and bacteriologically that clean wool cloth and wads from shot gun shells were the sources of the gas-producing organisms in clinical cases of gas gangrene. Clean wool samples and shot gun shell wads were cultured at the same time and the *Bacillus welchii* was demonstrated in each culture.

The following experiments were performed on animals either to refute or substantiate these clinical and bacteriological observations. Five dogs were used. The right and left thighs were shaved over the flexor group of muscles, thoroughly washed with soap and water, and followed by alcohol and iodine. Incisions were made in both thighs through skin and *fascia lata* down to the muscles. One muscle belly of the flexor group was then traumatized by crushing forceps. Pieces of wool cloth were introduced into this traumatized muscle of the left thigh and wads from shot gun shells introduced into the traumatized muscle of the right thigh. The *fascia lata* was then closed over the foreign body in the muscle and the skin was closed with continuous silk suture.

Nineteen hours after the introduction of the foreign bodies, the dogs showed the development of gas bacillus infection in each thigh. The presence of gas increased in 40% of the animals and decreased in 60%. The decrease in the amount of gas in 3 of the animals was due to rupture of the wound, which allowed the gas to escape.

Roentgenograms were taken at 19, 28, 41, and 51 hours respectively of both legs. In each instance, gas was demonstrated in the tissues. There was an increase in the amount of gas in the tissues in each instance until the wound ruptured in 3 of the animals, which allowed the gas to escape producing radiographic evidence of the decrease in the amount of gas present. Bacteriological study of

[1] Gage, I. M., *Am. J. Surg.*, 1926, **1**, 177.

the wounds revealed the presence of gas bacilli *(B. welchii)* in each animal.

The accidental contamination of traumatized wounds, either by clean wool cloth or wads from shot gun shells will result in the development of gas bacillus infection.

6146

Enzymes in the Alimentary Canal of Mosquito Larvae.

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An attempt has been made to determine the presence of enzymes in the digestive tract of certain species of mosquito larvae. Phillips[1] and Bertholf[2] demonstrated the presence of enzymes in the honey-bee, both larvae and adults, by feeding them on solutions of chemically pure carbohydrates and comparing the length of life in contrast to controls fed on water alone. By this method they hoped to eliminate the interference of any enzyme from the tissues surrounding the gut, a difficult matter in isolation techniques. However these workers did not exclude microorganisms from the alimentary canal and it is conceivable that organisms hydrolysed the carbohydrates which were able to maintain the insects alive for considerable periods.

The writer applied this method to *culicid* larvae and endeavored to exclude bacteria, etc., by sterilizing all media, either by autoclaving or filtering. Mosquito eggs disinfected in Hexyl Resorcinol (Hinman[3]) were introduced into solutions of chemically pure compounds, dissolved in a modified Ringer's Solution and incubated at a suitable temperature. Soluble starch, sucrose, galactose, xylose, levulose, lactose, maltose, glycogen, creatinine, cystine, tyrosine, a mixture of tyrosine and glycogen, a mixture of sucrose and tyrosine, have all been used. Solutions varied in strength from 0.1% to 1.0%. The results of these experiments have been rather inconclusive owing to inconsistency when repetitions were made. Checks

* National Research Fellow in the Biological Sciences.

1 Phillips, E. F., *J. Agric. Res.*, 1927, **35**, 385.

2 Bertholf, L. M., *J. Agric. Res.*, 1927, **35**, 429.

3 Hinman, E. H., *Am. J. Trop. Med.*, 1932, **12**, in press.

consisted of the sterilized Ringer's Solution as a medium. Larvae in a few cases were able to live for a significantly longer time in soluble starch, sucrose, xylose, glycogen, tyrosine, and cystine than in the checks.

Owing to these indefinite results, an attempt was made to isolate enzymes from the gut of the larvae of *Aedes aegypti* Linn and *Culex quinquefasciatus* Say. The intestinal tracts were dissected out, placed in 50% glycerin and stored in lots of 200 each. Before use these were finely ground up in an agate mortar. Technique in the main has been modified after Wigglesworth[4, 5] and Swingle.[6] Positive reactions to date have resulted in tests for amylase, invertase (sucrase), xylanase, and a protease acting in alkaline medium. Negative results were obtained in tests for maltase, lactase and a protease acting in acid medium. It is quite probable that a lipase is present in both of these species.

A comparison of the results of the rearing experiments with those of the isolation methods indicate that starch, sucrose and xylose, in certain instances, supported larval life for a significant period and that enzymes for the hydrolysis of these carbohydrates were detected in the digestive tracts of larvae. On the other hand, tests with maltose and lactose were negative according to both methods.

6147

Effect of Human Blood Serum on the Toxicity of Bile Salts.*

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Approximately two dozen white mice were used in these experiments. The effect on the toxicity of bile salts when injected intraperitoneally was observed, using normal saline and blood serum as vehicles. The lethal dose of stock bile salts[1, 2] dissolved in normal saline had previously been found to be 0.009 gm. This product

[4] Wigglesworth, V. B., *Biochem. J.*, 1927, **21**, 797.

[5] Wigglesworth, V. B., *Biochem. J.*, 1928, **22**, 150.

[6] Swingle, H. S., *Ann. Ent. Soc. America*, 1928, **21**, 469.

* This work was made possible through a grant of the David Trautman Schwartz Research Fund and the Touro Infirmary of New Orleans.

[1] Merck & Co., "Sodium Taurocholate".

[2] Williams, J. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 637.

contained proteins, etc., which were removed, since they might influence the results. The procedure consisted of cleaning with chloroform and ether and dissolving the solid matter in alcohol. The alcoholic solution cleared by standing was pipetted off, filtered, and evaporated and the residue dried in a hot air oven. The residue was then immediately weighed so as to obviate as much as possible the adsorption of water which factor would necessarily influence the weight.

The solutions when made up contained 0.001 and 0.002 gm. of bile salts to the cc. Their slight variation in acidity was not sufficiently marked in the author's experience to influence the results. The surface tension estimated by the drop method for the serum and saline salt mixtures was virtually the same for like concentrations of the salt.

When normal saline was used as a vehicle 0.009 gm. of bile salts were found lethal as in previous experiments.[1] When fresh human serum (not older than 3 hours) was used in a like capacity, at least twice the amount was necessary to insure a fatal outcome. Thirty-six hours were considered a logical time over which to read results, especially since animals which did not die within that time survived.

We observed that human serum is protective toward this toxic product. It is questioned that this is due to the presence of a developed resistance on the part of the body. The author by repeated injections has attempted to observe such a development of resistance in rabbits. Normal saline was used as a vehicle with the impure salt as solvent. The 5 rabbits used died in the process of the experiment although the dose was not increased. This would indicate, if anything, an accumulative effect. However, this list of experiments is not sufficiently great to draw conclusions and work on a larger number of animals is necessary. In addition purified salt may give better results since allergy might have played a part.

In explanation, it is thought that there is alteration of the disperse system of the blood serum. Possibly the bile salt with its greater surface tension adheres as a film to the serum molecule and in this manner delays the absorption of the toxic moiety, so that the body is at no time exposed to a lethal dose when less than 0.02 gm. is given. It is logical that the serum may also possess a neutralizing effect. This short piece of work and its discussion brings to the fore medicated serums and suggests the possibility of their use other than in the field of spinal lues.

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Effect of Human Blood Serum on Bile Salt Hemolysis.*

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In previous reports[1, 2] the effect of blood serum on the toxicity of bile salts injected intraperitoneally into the white mouse and the hemolytic effect of these salts dissolved in normal saline for red blood cells were observed. It was found that hemolysis occurred in dilutions of 1:200 to 1:6400. This large variation in hemolytic range might have been due in part to exposure of the cells under diverse circumstances of collection and in part to the heterogeneous type of individual from whom they were collected.

The present report concerns the effects of blood serum on hemolysis by bile salts after the stock product had been purified as explained in a previous article.[1] Different concentrations of this purified salt were made up in normal saline and in fresh human serum (not older than 3 hours). A drop of fresh homologous blood was then added to the saline and serum dilutions and the results observed in 3 and 24 hours. The hydrogen ion concentrations of the solutions were computed but the variation was not sufficient to influence the results. The surface tension of the several solutions of like concentration of the salt was taken by the drop method and this variation was found in each instance to be so slight that at least by the present perfection of technique estimation of the small difference was impossible. The tubes in which the solutions were mixed were allowed to stand at room temperature. Approximately 24 bloods were tested.

When human serum was used as the vehicle and readings made in 3 hours, complete hemolysis occurred in the tubes containing 2.5% bile salts, partial hemolysis in tubes containing 1.25%, and no hemolysis in those containing 0.625%. When normal saline was used as a vehicle complete hemolysis occurred in all tubes containing 0.625% bile salts, in ½ of the tubes containing 0.3125% and ⅓ of the tubes containing 0.10625%, partial hemolysis in ½ of the tubes containing 0.3125%, and ⅓ of the tubes containing 0.10625% and no hemolysis in ⅓ of the tubes

* This work was made possible through a grant of the David Trautman Schwartz Research Fund and the Touro Infirmary of New Orleans.

1 Williams, J. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, —.

2 Williams, J. W., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 913.

containing 0.10625%, and all tubes containing 0.053125%. After standing 24 hours there was a shift of hemolysis, in some instances several dilutions higher when normal saline was used as a vehicle. On the other hand, with the blood serum vehicle no such marked shift was noted.

The foregoing results indicate that both white mice and red cells are protected by blood serum to a considerable degree from the toxic effects of bile salts. This substantiates our belief that either the serum has a neutralizing effect or that the serum molecules by affording a surface capable of being coated by bile salts diverts in part or toto this substance from the red cell and thus prevents or delays hemolysis.

6149

Peripheral Course of Sensory Nerves Supplying Arteries of Lower Extremity.

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Lumbar sympathetic gangliectomy abolishes the reflex vascular spasm, the central feature of Raynaud's disease. The immediate relief of the accompanying pain is so striking that it has led clinical observers to conclude that some type of sensory nerve to the lower extremity is sectioned in the course of the sympathectomy. This view is strengthened by the experiments of Johnson[1] and of Kuntz and Farnsworth,[2] which demonstrated that certain of the dorsal root components of the lower dorsal and upper lumbar spinal nerves pass to the lumbosacral sympathetic trunks and are distributed from them to the lumbosacral plexus.

During the course of experiments in arterial visualization, we noted that cats under sodium amytal anesthesia reacted in a characteristic manner when a concentrated solution of sodium iodide was injected into the femoral artery. The entire body stiffened with the legs straining at the leashes; hyperpnea; dilatation of the pupils; tossing of the head accompanied by vocalization, the outcry possess-

[1] Johnson, S. E., *J. Comp. Neurol.*, 1921, **33**, 85.

[2] Kuntz, A., and Farnsworth, D. I., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 808.

ing sometimes a plaintive note and sometimes a note of anger. This reaction of the anesthetized animal is identical with the external manifestation of the perception of acute pain during conscious states. It is not accompanied by subjective perception of pain, for Woodworth and Sherrington[3] showed that such "pseud-affective" reactions persist after ablation of the cerebrum and diencephalon. It results, we may presume, from afferent impulses which, were the function of the brain intact, would evoke "pain".

We made use of this "pseud-affective" reaction of the amytalized cat in an attempt to determine the course and termination of the "pain" fibers stimulated by the intra-arterial injection of the sodium iodide. When the iodide is confined to the trunk artery by the ligation of its branches, no reaction occurs. Therefore the fibers do not terminate in the lining of the large vessels. (Odermatt[4] arrived at the same conclusion as regards other irritating solutions.) Such an experiment lacks conviction when applied to the femoral artery where ligation of the numerous branches requires such isolation of the vessel in its bed as conceivably to interrupt its nerve supply. The left subclavian artery of the cat, however, courses for nearly 2 inches before branching. When it is ligated proximal to this branching, an injection with sodium iodide evokes no reaction whatever.

The experimental blocking of the femoral arterioles with lycopodium spores delays the appearance of the reaction considerably. This delay suggests that the fibers terminate either in the capillaries or in adjacent cellular tissues to which the iodide may diffuse. We have not yet succeeded in localizing further the nerve endings upon which the irritating solution acts. The end-organs of cutaneous sensibility, however, are not involved, since the reaction occurs upon injection of arteries of purely visceral distribution such as the hepatic artery and the splenic.

Quite definite information has been obtained regarding the peripheral course of the fibers conveying the impulses in question. Unilateral or bilateral resection of the entire lumbar sympathetic chain neither prevents nor modifies the reaction. In contrast, the division of the branches of the ipsolateral lumbosacral plexus prevents the occurrence of any reaction whatever. As a parallel, if one lumbar sympathetic chain is removed and the branches of the contralateral lumbosacral plexus sectioned, a typical "pseud-affective" reaction occurs when the femoral artery of the sympathectomized

[3] Woodworth, R. S., and Sherrington, C. S., *J. Physiol.*, 1904, **31**, 234.

[4] Odermatt, W., *Bruns' Beiträge z. klin. Chir.*, 1922, **127**, 1.

side is injected, whereas no reaction results from injection of the opposite vessel. Therefore it is obvious that the afferent fibers concerned in the reaction reach the cord by way of the peripheral spinal nerves and not by way of the sympathetic chain. This finding is in agreement with that of Bradford Cannon,[5] who, using buried electrodes, could elicit no sign of pain upon stimulation of the lumbar sympathetic chain in the unanesthetized animal.

It is concluded that the intra-arterial injection of irritating solutions causes pain through the stimulation of afferent nerve endings located in or in close association with the finer arterial branchings. Lumbar sympathectomy does not abolish pain of this origin and relieves only pain arising directly or indirectly from arterial spasm.

[5] Cannon, Bradford, 1932, personal communication.

Iowa Section.

State University of Iowa, April 19, 1932.

6150

Effect of Adrenalin on Rate of Blood Transfer in Parabiotic Rats.

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In work with parabiotic rats it becomes necessary to know something of the extent of blood transfer from one animal to its parabiont. Several authors (Martins,[1] Kallas,[2] Kawashima,[3] and others) have proven blood exchange through physiological or chemical tests, none of which, however, seem quantitative. The author[4] found much variation in different pairs and in the same pair at different times. The present work is an attempt to discover possible factors involved in such variations.

The dye, 1% Brilliant Vital Red (Evans), was used in Locke's solution. The adrenalin (hydrochloride compound of 1:1000 strength, preserved in 1% chloretone) was furnished gratis by the Parke, Davis and Company Research Laboratories. Chloretone was removed prior to use. The volume of adrenalin injected was 0.025 cc. and of dye 0.2 cc.

Control tests were run on each pair of rats by the method previously described (loc. cit.). The percentages expressed in this paper are based on the relative amounts of dye in each of the 2 animals at the end of one hour.

Experiment I. Dye and adrenalin were injected simultaneously in the tail vein of the left twin (all injections made directly into the tail vein). Immediately afterward adrenalin alone was injected in the right animal.

Experiment II. The right animal of each pair was not injected

[1] Martins, Th., *Compt. Rend. Soc. Biol.*, 1929, **102**, 614.

[2] Kallas, H., *Compt. Rend. Soc. Biol.*, 1929, **102**, 280.

[3] Kawashima, Hiroshi, *J. Fac. Science, U. of Tokyo*, 1931, **2**, 141.

[4] Hill, Robert T., Proc. Soc. Exp. Biol. and Med., 1931, **28**, 592.

at all. Adrenalin mixed with dye was injected into the left member.

Experiment III. Adrenalin was injected in the right animal, the left twin receiving dye solution only.

Experiment IV. Dye was injected into the left twin at an interval of one minute after the same animal had received adrenalin. The right animal was not injected at all. The results of these experiments are expressed in Table I.

TABLE I.
Percentage of Dye in the Uninjected Animal at the End of One Hour.

	Control No Adr.	Adr. to both	Adr. to left	Adr. to right	Adr. to left 1 min. before dye
	%	%	%	%	%
	27.1	12.9	11.8	5.1	10.9
	29.1	5.3	7.3	5.7	6.8
	20.8	9.3	9.3	9.6	7.1
	27.7	5.4	6.7	4.9	8.3
	32.2	8.8	10.3	8.5	6.4
	31.1	8.3	9.7	7.4	10.1
	24.3	15.8	11.2	10.5	died
	25.0	10.9	9.9	10.7	10.8
	28.2	11.0	9.0	8.4	12.9
		9.8	3.7	4.3	
	32.8	9.8	11.3	12.9	8.7
	28.2	9.3	4.2	2.2	7.0
	21.9	6.3	6.2	2.5	
	21.5	11.7	8.7	8.5	9.6
	17.3	6.0	13.3	15.0	
Average:	26.4	9.1	8.9	7.8	8.6

Note: Average of all tests combined is 8.6% (with adrenalin) which is 32.2% of the average of control tests.

The results of these blood transfer tests made with the use of adrenalin, regardless of whether the adrenalin is given to one or both members of a pair, all show a very close correlation. The average of the readings from any one experiment does not differ greatly from the average of any other set of experiments. However, the average of the combined results of the adrenalin tests is only about 33% of the average of the control measurements. From the closer correlation in the results obtained in this set of experiments than in those of the control set or of previous tests, it becomes quite apparent that the rate of blood exchange seems, under normal conditions, controlled more or less by reason of a greater or lesser amount of adrenalin being released into the blood stream during and immediately previous to the experiment. Individual differences in excitability of the animals may play an important part in the amount of adrenalin released. This reduction in the rate of transfer with the use of adrenalin seems very significant.

6151

Determination of Formic, Acetic and Propionic Acids in Fermenting Mixtures.

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The procedure involves the use of the partition method[1, 2] for the determination of organic acids, in conjunction with the separate determination of the formic acid by oxidation with mercuric oxide. The determination of the formic acid in this manner is necessary because the partition method will not serve to determine formic in the presence of acetic acid. By means of a nomogram the formic acid determination is read off and connected by means of a straight edge to the partition reading; the percentages of acetic and propionic acids are read off on the appropriate lines on the nomogram.

The acidity of the acid distillate should be adjusted to approximately 0.03 normal.

Two hundred cc. of the mixture are boiled for half an hour with about 3 gm. of mercuric oxide in a flask. The carbon dioxide liberated during the process is determined, and the formic acid calculated and expressed in terms of normality as percent of total acid present.

Fifty-five cc. of the acid mixture are shaken for 1½ minutes in a separatory funnel with 25 cc. of ethyl ether. Fifty cubic centimeters of the aqueous phase are then removed and titrated. The cc. of alkali required to titrate these 50 cc. of the acid divided by the cc. of alkali required to neutralize 50 cc. of the acid before partition gives the partition constant, K.

Constants established for each acid are: for formic $K = 84.5$, for acetic $K = 82.1$, and for propionic $K = 58.8$.

Expressing the percent of formic, acetic, and propionic acid present by F, A, and P respectively the following equations are set up:

$$\begin{aligned} 0.845\,F + 0.821\,A + 0.588\,P &= K \\ F + A + P &= 100 \end{aligned}$$

or since F is determined separately,

$$\begin{aligned} 0.821\,A + 0.588\,P &= K - 0.845\,F \\ A + P &= 100 - F \end{aligned}$$

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[1] Werkman, C. H., *Ind. Eng. Chem.*, 1930, **2**, 302.

[2] Osburn, O. L., and Werkman, C. H., *Ind. Eng. Chem.*, 1931, **3**, 264.

Solving these equations for A and P in terms of K and F we have:

$$A = 4.291\ K - 1.103\ F - 252.36, \text{ and}$$
$$P = 352.36 + 0.103\ F - 4.291\ K$$

The percent of each acid is expressed as a true normality percent, *i. e.*, the number of cubic centimeters of the normal acid per 100 cc. of total normal acid.

The presence of acids other than the 3 mentioned may be detected by partitioning 60 cc. of the acids with 100 cc. of ether and establishing constants as above. If the results of such a partition indicate a different ratio of propionic to acetic acid, then some foreign acid is indicated. The individual constants for 60 cc. of the acid mixture and 100 cc. of ethyl ether are: for formic $K = 63.5$, for acetic $K = 58.5$, and for propionic $K = 27.16$.

Missouri Section.

Washington University School of Medicine, April 13, 1932.

6152

Responses of Axons to Brief Shocks.

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In a previous paper[1] a range of latencies was postulated in the response of nerve fibers to short induction shocks. Using a 5-panel amplifier in conjunction with the von Ardenne cathode ray oscillograph, permitting the photographic recording of input potential changes significant to one microvolt and of time to 0.01σ, we are now able to secure by an inertialess method records of the potential changes in nerve resulting from the activity of a single fiber.

The monophasic axon spike varies in amplitude from 10 to 300 microvolts, depending largely on the mass of inactive shunting tissue. In the α range the rising phase at 16°C. varies in different experiments from 0.3 to 0.7σ. The falling phase may be as short as 1.0σ. The β spike seems to have comparable magnitudes, although statistically they may be a trifle longer. Spikes of B fibers in a gray ramus had a crest time, at 16°C., of 1.4σ. Shapes and time functions of the axon spikes vary considerably in different experiments. The most important modifying factors seem to be the condition of the reacting fiber, the depth of tissue between it and the lead electrode, branches or adherent material between leads, potentials from remote active fibers, extraneous disturbances, the diphasic artifact, and the type of fiber most favorably placed in relation to the stimulating electrode.

The usual axon spike shows a short inception phase, most marked in nerves with sheaths, which we believe represents in part the potential change led through inactive tissue, ahead of the arrival of the excitatory process over the lead. Extremely small nerves may give axon spikes which show a vanishingly small period of incep-

[1] Erlanger, J., and Blair, E. A., *Am. J. Physiol.*, 1931, **99**, 129.

tion; the rise then begins linearly, the crest is sharp, and the visible decline ends abruptly. We believe that these curvilinear, triangular spikes are the least deformed of any we have recorded.

Quick shocks (duration less than 0.04σ) which appear to be uniform when subjected to physical and physiological tests, may be adjusted so that only an occasional response of a single axon occurs, indicating that extirpated fibers are subject to spontaneous changes in irritability. The interval between the delivery of the shock and the arrival of the spike at the lead also varies spontaneously. In a typical experiment at 16°C., employing uniform shocks producing only occasional single axon responses, shock-spike intervals varied spontaneously over a range of 0.4σ. Increase in strength tended to decrease the spontaneous range and shorten the average shock-spike interval. Change of strength from just threshold to 3 times that value brought this spike still earlier so that the extreme range of its latency amounted to more than 0.7σ.

Submaximal spikes recorded at the site of stimulation have been assumed in the past to represent the time functions of undispersed axon spikes. In a typical experiment at 16°C. a composite α spike recored at the site of stimulation had an overall time to maximum of 0.94σ. The time to maximum of the most irritable component α spike was nearly 0.4σ. Since fibers of much lower irritability than any involved above have spikes which are little longer than those of α, this difference cannot be attributed to the presence of significantly longer axon spikes; it must be assigned to fibers responding with latencies ranging in this case from less than 0.2σ up to 0.5σ or more.

A conducted action potential involving few units is of irregular height and shape. The most irritable fiber responds consistently with an almost uniform short latent period, and its spike is usually the first to arrive at the lead; some of the slower, but threshold, fibers respond only occasionally with long, variable latent periods. Although the shock-spike time is fairly constant, the pictures are constantly varying. The regular configuration of spike responses previously published is due to the smoothness of the sum of the component axon potentials despite the entrance of units after the crest is reached and despite a certain amount of play of the fibers responding.

6153

Fibers in Mixed Nerves and Their Dorsal Roots Responsible for Pain.

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It has been shown that fibers which differ in anatomical type, for instance myelinated and unmyelinated fibers, when stimulated, give electrical potentials which differ characteristically (Bishop and Heinbecker[1]). The potential picture recorded from a mixed nerve by means of the oscillograph thus serves as an index of the nerve's fiber content. When these potentials are recorded from a nerve still in the body, the correlation between potential record and the response given by the animal as a whole serves to identify the fibers responsible for accomplishing a specific type of activity. The potential record from a nerve, like the electrocardiogram, thus becomes an index of physiological function. We here present data to identify a group of nerve fibers which convey impulses resulting in painful sensations.

Autonomic fibers in mixed nerves can be recognized by the fact that they are slow-acting, and myelinated autonomics can be differentiated from unmyelinated by thresholds and conduction rates. Of somatic fibers, 2 groups can be distinguished, both myelinated, one of which conducts more rapidly than the other. The slower includes the so-called "visceral afferents". There are thus 4 groups recognizable, general somatic, visceral afferent, myelinated autonomic and unmyelinated autonomic. Pain is mediated by fibers of the second group, of which the visceral afferent is the type. Whether pain is also conveyed by unmyelinated fibers in mixed nerves we have been unable to determine so far, since stimulation of the myelinated fibers causes so much pain that an increase of sensation which might result from stimulation of unmyelinated fibers of higher threshold cannot be detected.

A first experiment was conducted in a conscious human. A branch of the saphenous nerve was dissected out after novocaine anesthesia to the skin only. The threshold stimulus required to cause pain was determined. It was found that at this strength of stimulus at least 8 stimuli were required before pain was experienced. With stronger stimulation fewer stimuli were required. Here, as

[1] Bishop, G. H., and Heinbecker, P., *Am. J. Physiol.*, 1930, **94**, 170.

in the study of sympathetic nerve fibers to the eye, (Bishop and Heinbecker[2]) the number of fiber stimuli rather than the frequency or intensity alone determined the degree of effect. The fiber type which was being stimulated when pain was felt was determined by a potential analysis of a branch of the external popliteal nerve of the same leg (after amputation) of corresponding size and with the same stimulating circuit. It was found that the threshold stimulus required to elicit pain was just adequate to elicit a threshold response of the second potential, from myelinated fibers of the visceral afferent type. These fibers are smaller and more thinly myelinated than the large thickly-myelinated sensory and motor fibers found in a mixed trunk. They include the smallest somatic myelinated fibers. It is important to note that the threshold for reflex muscular responses was the same as the threshold for the sensation of pain.

Experiments were carried out in which an electrode shielded except at the tip was inserted through the skin over a branch of the median cutaneous nerve of the forearm in six conscious humans. The indifferent electrode was placed on the skin 3 or 4 cm. distant and kept moist. The ratio of the threshold for tactile sensations to the threshold required to elicit pain was found to be 1:5 or 1:6. This ratio is of a similar order to that determined for the thresholds of the first and second complexes with fast properties in mixed human nerve trunks. Somewhat similar experiments were carried out in the dog. Here a distal portion of the saphenous nerve was dissected out under general anesthesia. Stimulating and recording electrodes were applied to the nerve trunk after the animal was allowed to recover from the anesthesia. The ratio of thresholds for the first and second potential complexes was found to be 1:5 to 1:6. Pain was again experienced when the stimulus was adequate to elicit the second potential complex. The threshold for muscular reflex response was found the same as the threshold for pain.

Trunks composing the sciatic nerve of the dog and cat with their attached roots were dissected out in order to trace the second potential complex, found to be associated with the sensation of pain, into the spinal cord. On stimulating the nerve trunk, this potential was recorded from the dorsal roots.

The thresholds for motor responses in spinal roots and in nerve trunks are of the same order, and the ratios of stimulus strength for direct motor and reflex responses are the same. Cross sections of

[2] Bishop, G. H., and Heinbecker, P., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 682.

osmicated nerves and roots both show the fibers inferred to correspond to the second potential associated with pain.

Both functional and histological evidence therefore support the conclusion that the impulses giving rise to the sensation of pain are mediated by myelinated fibers coursing *via* the dorsal roots, having the properties of the visceral afferent group.

6154

Observations on "Encystment Cycle" of Endamoeba histolytica in a Carrier.

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The stools of a human carrier of *E. histolytica* were studied daily for 215 days to determine (1) the presence of encystment cycle, (2) the development and viability of immature cysts outside of the body, effects of changes in temperature and moisture, (3) the relative proportions of uni-nucleate cysts at various periods of encystment cycle, and (4) correlation, if any, between consistency of stools and number of discharged cysts.

The number of cysts per gram of stool was calculated according to the method previously described[1] and if cysts were very few, according to a modification of Lane's method[2] for counting hookworm eggs. All negative findings were verified by Rivas' modification[3] of ether-acetic acid concentration method, and also by the culture method recently reported.[4] Gram iodin solution was used in determining the number of nuclei present in samples of cysts examined.

Observations. 1. *Encystment Cycle.* There was a definite cycle of encystment in *E. histolytica* as has been observed in giardias by Boeck,[5] Kofoid and others[6] as well as by the author.[1] There was an apparent periodicity in appearance of the maximum number of

[1] Tsuchiya, H., *Am. J. Hyg.*, 1931, **13**, 544.

[2] Lane, C., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1924, **17**, 407.

[3] Rivas, D., *Am. J. Trop. Med.*, 1928, **8**, 63.

[4] Tsuchiya, H., PROC. SOC. EXP. BIOL. AND MED., 1931, **26**, 347.

[5] Boeck, W. C., *Uni. Col. Pub. Zool.*, 1922, **20**, 199.

[6] Kofoid, C. A., Boeck, W. C., Minnich, D. E., and Rogers, J. H., *J. Med. Res.*, 1919, **39**, 293.

cysts in each cycle, and the durations of such cycles ranged from 8 to 10 days, the average being 9.2 days. The number of cysts fluctuated from day to day with the maximum of approximately 1,250,000 per gram of stool. In most instances a peak in number was followed by a gradual diminution, and this was subsequently followed by a gradual rise until another peak was reached. However, when a negative period (the complete disappearance of cysts from stool) preceded a cycle, there was observed a sudden rise in the number of cysts.

2. *Development and Viability of Immature Cysts.* Contrary to the general belief that immature cysts were incapable of development outside of the body, it was found that under favorable environmental conditions such as an optimum temperature and a sufficient moisture, the cysts may develop to maturity *in vitro*. Thus uni- and bi-nucleate cysts developed into maturity quite readily at room temperature (22°C.) as observed by Hegner, Johnson and Stabler,[7] and in a refrigerator temperature (5°C.), while at 37°C. the development was extremely poor. The viability of such cysts was found to be greatest in a refrigerator (28-35 days for washed cysts and 9-11 days for cysts in original stools). At room temperature, washed cysts remained viable for 7-9 days, while in original stools for 3 to 6 days. The longevity of washed cysts in a refrigerator may be accounted for by the presence of fewer bacteria and the retardation of their growth.

3. *Relative Proportions of Uni-nucleate Cysts in Cycle.* The greater the total number of cysts, the greater was that of uninucleate cysts, and as the number of cysts became less and less in a cycle, there was also a gradual diminution in the proportion of uninucleate cysts. This seems to indicate that a peak in the number of cysts represents the beginning rather than the middle of an encystment cycle.

4. *Correlation Between Consistency of Stools and Number of Cysts.* The factors which influence the consistency of stools seem to have no effect upon the encystment cycle, and thus the principle governing the latter may be considered as strictly biological in nature.

Comments. The above observations emphasize the importance of frequent examinations of stools in suspected cases of the amoebic infections. Judging from the manner in which cysts were discharged from day to day, it is suggested that an optimum result may be obtained by examining stools on alternate days for a longer

[7] Hegner, R., Johnson, C. M., and Stabler, R. M., *Am. J. Hyg.*, 1932, **15**, 394.

period rather than on 6 consecutive days as advocated by Dobell.[8] The viability of immature cysts at low temperature suggests the possibility that such cysts may play an important rôle in the spread of the infection in cold season, and incidentally explains why the incidence of the carriers is relatively frequent in north temperate zones.

6155

Further Studies of the Ovaries of Monkeys.

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From the University of Missouri School of Medicine.

During the course of removal of ovaries from monkeys to prepare them as test animals for experiments with ovarian hormones, analyses of the ovarian follicular hormone (theelin) content have been made of individual follicles and corpora lutea. Ovariectomized mice have been used as test animals.

Solid tissues have been implanted in small pieces or simultaneous implants of solid tissues and injections of liquor folliculi have been made. Where possible ova have been recovered to check the normality or atresia of the follicles.

Similar series of experiments have been in progress with follicles of different sizes from pig ovaries, the test being made in ovariectomized rats.

Although additional tests must be added before publication of the data, enough evidence has already been obtained to justify the conclusion that theelin reaches a higher concentration in the follicles of monkeys than in the follicles of the pig. This is to be expected since the pig is a litter-bearing animal.

[8] Dobell, C., *Med. Res. C. Sp. Rep. Ser.*, 1921, **59**, 71.

Pacific Coast Section.

University of California, April 23, 1932.

6156

Ion Intake in Valonia as Affected by HCl and CO_2.*

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Living cells of *Valonia ventricosa* J. G. Ag. were placed in sea water acidulated to pH 5.2 with CO_2 or HCl. After 3 or 12 hours in these solutions groups of 7-11 cells were removed, and the K, Na, and Cl concentrations in the sap extracted from them were determined, and compared with the mean of those in the sap from 3 similar groups of untreated cells. The cells in each sample were selected so as to be of similar approximately uniform size. Excess CO_2 was removed from the sea water-HCl mixture before using it.

In no case was there any clearly demonstrated change during the first 3 hours, although the low value of K at this time in the HCl experiments may be significant.

Cells kept 12 hours in sea water-HCl mixture showed a 2.7% increase in K, and a 20% decrease in Na. These correspond to changes of 14 and 10 milli-equivalents per liter, respectively, and are more than double the mean variation between the individual groups of control cells. There was no significant change in Cl.

Cells kept 12 hours in sea water-CO_2 mixtures showed a 1.7% decrease in K and a 64% increase in Na. These corresponded to changes of 9 and 32 milli-equivalents per liter, respectively. The change in K, although too small to be considered significant when evaluated alone, probably represents a real change, since it accompanies an indisputable increase in Na. There was again no change in Cl.

Thus HCl led to an increase in K and a decrease in Na, while CO_2 had the opposite effect. The sea water acidulated with CO_2 differed

* This work was aided by a grant from the Board of Research of the University of California.

from that acidulated with HCl principally in its higher content of CO_2 and HCO'_3: there was no significant difference in pH or in Cl' content. Experiments of M. M. Brooks[1,2] indicate that similar differences in CO_2 and HCO'_3 and likenesses of pH probably prevailed in the sap of the cells throughout most of the experiment. The pH of the sap was observed to be close to that of the surrounding solution at the end of the present experiments also. These facts alone do not lead to any explanation of the observed changes of K and Na concentrations in the sap.

These changes can be explained in accordance with the writer's hypothesis of ion accumulation as a non-equilibrium condition involving ionic exchange,[3] if one assumes that decreased pH leads to a transient decrease in permeability to cations, which, at least in the presence of abnormally large concentrations of CO_2 (and) (or) HCO'_3, soon gives place to an increased permeability which ends in injury and death. Such a succession of changes in permeability to ions as a result of decreased pH has been shown for *Laminaria* by Osterhout.[4]

During the phase of decreased permeability to cations the rate of intake of Na would be retarded more than that of K, while those of Cl and H_2O would remain intermediate. K would therefore enter faster than H_2O and increase in concentration, while Na would enter slower than H_2O and decrease in concentration. This would lead to the situation found after 12 hours immersion of the cells in sea water + HCl. In sea water + CO_2, on the other hand, the secondary increase in permeability would have become established considerably before the end of the 12 hours, so that its effects, namely, an increase in the rates of intake of Na > Cl, H_2O > K, would have led to changes opposite to those just described, and resulted in conditions such as were actually found.

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[1] Brooks, M. M., *Pub. Health Reports*, 1923, **38**, 1449.

[2] Brooks, M. M., *Pub. Health Reports*, 1923, **38**, 1470.

[3] Brooks, S. C., *Protoplasma*, 1928, **8**, 389.

[4] Osterhout, W. J. V., *J. Biol. Chem.*, 1914, **19**, 493.

6157

Effect of Sodium Fluoride on Lactic Acid Production by Streptobacterium Casei.

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Fluorides, like the halogenated acetic acids, inhibit the formation of lactic acid from hexose or glycogen in biological systems by causing the formation of stable hexose esters.[1] We have reported observations on the course of iodoacetate poisoning in *Streptobacterium casei*,[2] and can now add to these the course of events in fluoride poisoning.

The experimental procedure was the same as previously reported[2] except that various concentrations of sodium fluoride instead of iodoacetate, made up in the suspension medium, were placed in the sidearms of the Warburg vessels.

As in iodoacetate poisoning there was a brief latent period on addition of fluoride. However, the rest of the picture was quite different. Whereas inhibition was observed in all cases with iodoacetate, in concentrations ranging from 0.0004% to 4.0%, with fluoride we found that concentrations up to 0.08% actually stimulated lactic acid production. At a concentration of 0.10%, the lactic acid-time curve was approximately the same as that of the control. Increasing the fluoride concentration up to 0.18% caused small decreases in the rate of lactic acid production, then there was a sharp decrease in this rate when the fluoride concentration reached 0.20%. Little more inhibition resulted from a fluoride concentration up to 0.5%.

Unlike the smooth continuous decrease in rates observed with iodoacetate, fluoride poisoning causes a rather sharp break in the rate of lactic acid production, after which the rate remains constant for several hours, at a characteristic value for each fluoride concentration.

As in previous experiments, lactic acid production in the control vessels was carried out at a constant rate for some 7 hours, the duration of the experiments.

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[1] Barrenscheen, H. K., Braun, K., and Dreguss, M., *Biochem. Z.*, 1931, **240**, 381.

[2] Field, J., and Field, S. M., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 733.

6158

Observations on a Delayed Skin Reaction to Cold.

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Following the application of ice to the skin of the forearm for 2 minutes a late local reaction at the site of application has been noted. The reaction appears after 18 to 24 hours, and varies from a slight erythema to a marked circumscribed local edema and erythema, with itching, burning and pain. The lesions persist from one to 5 or 6 days, the duration varying with the severity of the reaction.

Reactions have been noted in 10% of normals (60 subjects), 7% of patients with heart disease (77 subjects), 45% of patients with asthma or hay fever (20 subjects), 51% of patients with pulmonary tuberculosis (146 subjects), and 61% of diabetic patients (50 subjects). Severe reactions have occurred only in patients with pulmonary tuberculosis. Studies of the immediate behavior of the surface temperature show no difference between reacting and non-reacting subjects.

Immediate urticarial reaction to cold has been described by Duke[1] and others, and is analogous to the immediate reaction seen in protein skin tests on allergic subjects. The late reaction to cold here described corresponds to and resembles the delayed response of the tuberculin and other similar skin tests. This analogy, as well as the frequency of the reaction in the small group of allergic patients suggests that the mechanism of cutaneous hypersensitiveness to cold is closely related to that of other forms of skin hypersensitiveness.

The reaction may be regarded as an experimental chilblain. The high incidence of chilblains among patients with tuberculosis has been noted by Hallam.[2]

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[1] Duke, W. W., *Arch. Int. Med.*, 1930, **45**, 206.

[2] Hallam, R., *Brit. Med. J.*, 1931, **1**, 215.

6159

Isolation of Coccidioides Immitis (Stiles) from the Soil.

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The epidemiological studies on coccidioidal granuloma, recently summarized by Beck,[1] suggest the soil and the vegetation as the probable source of the fungus infection. The peculiar geographic distribution of the disease both in man and animals to the central and southern sections of the State of California with a predominance of the cases in Kern and Los Angeles Counties supports this contention. Although repeated attempts have been made to find the organism in nature the examinations, made with the aid of the customary bacteriologic technique, have thus far yielded negative results. The relatively slow growing atypical fungi are, as a rule, rapidly overgrown by the concomitant bacteria present in the soil and vegetations. Little progress could be made until a procedure had been developed which would concentrate, possibly enrich the stage of the mold (chlamydospore) supposed to exist in nature. In the course of detailed metabolism studies it was found that *Coccidioides immitis* satisfies its nitrogen and carbon requirements from relatively simple compounds. The medium, which gives selective cultures, has the following composition: Ammonium chloride 1 part, sodium acetate 1 part, equal mixture of KH_2PO_4 and K_2HPO_4 0.4 part and magnesium sulfate 0.01 part; distilled water 100 parts. While the majority of bacteria grow slowly or very poorly, *Coccidioides,* certain *Blastomyces* and *Aspergillus* varieties develop exceedingly well in the nutrient solution. Bacterial multiplication may be entirely suppressed and the purification of the fungus greatly facilitated by the addition of acriflavine in a concentration of 1:25,000. For several years the medium has been employed in the examination of clinical specimens (sputum, pus, blood, autopsy specimens, etc.) suspected to contain the mold. Samples of sputum, which contained very few typical double contoured fungoidal cells yet teeming with the usual respiratory and buccal flora, gave pure cultures of *Coccidioides immitis* in the dye culture medium.

Since *Coccidioides* survives the exposure to a 30-35% NaCl solution for upwards to 3 to 4 hours, a method has been developed which permits the concentration of the fungus spores from large samples of soil or vegetation. The procedure, which has repeatedly

[1] State of California Dept. of Public Health Special Bull., 1931, 57, 19.

yielded the pathogenic fungus from specimens of earth collected around the sleeping quarters at a ranch near Delano, Kern County, is as follows: The sample of dirt is mixed with a small amount of brine and triturated into a thick paste which in turn is permitted to percolate into a tall, narrow cylinder containing 30% salt solution. As the particulate matter passes through the long column of brine the bacterial and fungus spores are set free, and rise slowly to the surface of the liquid. After an exposure of the soil to the action of the brine for 3 hours the supernatant fluid is decanted and diluted one-half with sterile water and centrifuged. The sediment is either cultured in the selective medium or inoculated subcutaneously into guinea pigs.

Positive findings on the soil of the Delano ranch had been anticipated on account of the occurrence of 4 cases of Coccidioidal granuloma among the Filipino working crew (1, p. 25). The guinea pigs, injected subcutaneously with the soil sediment, developed either a generalized infection with miliary localization in the lungs, purulent epididymitis and lymphadenitis or merely temporary small abscesses at the site of inoculation. The pus contained the characteristic double contoured capsules with the endospores. Obviously, the mold, isolated from the soil, is highly virulent, and infects on primary inoculation. However, it is not unlikely that the variable susceptibility of the guinea pigs may, in part, have been responsible for the failure to detect the fungus in a few other samples. Experiments are now in progress to improve the pathogenicity tests and to demonstrate directly by special cultures or other methods, the resting stage, which permits the mold to survive for months in a desiccated soil specimen.

New York Meeting.

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6160

Precipitin Formation in S. Viridans Subacute Endocarditis.

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The sera of nineteen patients* with *S. viridans* sub-acute endocarditis have been studied for precipitins against protein fractions of *S. hemolyticus*,[1] *S. viridans*,[2] *Staph. aureus*,[2] and the group specific carbohydrate of *S. hemolyticus*. The *S. viridans* and *Staph. aureus* fractions were 5 and 7 years old, respectively, whereas the *S. hemolyticus* fractions were freshly prepared. All of the patients had the classical disease picture. Blood cultures were negative in 2 of the cases. These patients are to be studied further. The sera were collected at a time when the disease had been sufficiently advanced to justify the patient's admission to the hospital. On the basis of clinical history and the autopsy findings in some cases, 17 of the patients had rheumatic heart disease, and one of the remaining individuals had a normal heart at post-mortem, except for the fresh bacterial endocarditis.

The precipitin test was performed by adding 0.3 cc. of each serum to 0.3 cc. of the respective antigen and incubating the tubes in a water bath at 37°C. for 2 hours. The material was left in the ice-box over night and the initial reading was made in the morning. The tubes were then centrifuged at 1700 revolutions per minute for 10 minutes and a second reading was made.

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* We are indebted to Dr. J. D. Lyttle of the Babies' Hospital and to Dr. B. S. Oppenheimer and Dr. R. Ottenberg of the Mt. Sinai Hospital for some of these sera.

[1] Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1931, **54**, 515. Fraction K represents the portion extracted by 0.2 *N* NaOH from the residue from the *G* fraction.

[2] Lancefield, R. C., *J. Exp. Med.*, 1928, **47**, 481. These nucleoproteins were kindly supplied by Dr. Lancefield.

TABLE I.

Precipitin Formation in *Streptococcus Viridans* Subacute Endocarditis.

Serum No.	*S. hemolyticus* 19 D O.N.	*S. hemolyticus* 19 D C.	*S. hemolyticus* 18 K O.N.	*S. hemolyticus* 18 K C.	*S. hemolyticus* 8 C O.N.	*S. hemolyticus* 8 C C.	*S. viridans* O.N.	*S. viridans* C.	*Staph. aureus* O.N.	*Staph. aureus* C.	Serum and Salt Control O.N.	Serum and Salt Control C.
35	0	+	+	+	0	++	0	++	+	+	0	++
36	+	+	++	+	0	+	0	+	0	+	0	0
37	++	++	+	+	0	+	0	0	0	++	0	0
38	++	+	+	+	0	++	0	++	0	++	0	0
39	+++	+	+	+	0	++	++	+	0	+	0	0
40	++	+	+	+	0	0	++	+	0	++	0	0
41	+	+	+	+	0	+	+	+	0	+	0	0
42	0	+	+	+	0	0	+	+	0	++	0	0
43	+	+	+	+	0	0	0	+	0	++	0	0
44	++	+	0	+	0	0	0	0	0	++	0	0
45	+	+	+	+	0	+	++	+	0	0	0	0
80	0	+	+	+	+	+	+	+	0	0	0	0
269	+	+	+	+	0	+	0	+	0	0	0	0
270	++	+	+	+	0	+	+	++	0	0	0	0
*271	++	+	+	+	0	+	+	++	0	0	0	0
*272	++	+	+	+	0	+	+	++	0	0	0	0
341	0	+	0	0	0	0	0	+	0	+	0	0
358	++	+	0	+	0	0	0	+	0	0	0	0
375	++	+	0	+	0	0	+	+	0	0	0	0

Protein fractions and saline = 0. O.N. = Overnight reading. C = Centrifuged specimen reading.
19 D: Acetic acid precipitable *S. hemolyticus* protein extractable at neutrality; concentration 1:2,000.
18 K: Acetic acid precipitable *S. hemolyticus* protein extractable (after removal of less alkaline extracts) between pH 11 and 13.3; concentration 1:2,000.
8 C: Species specific polysaccharide, concentration 1:200,000.
S. viridans and *Staph. aureus* protein solutions, concentration of soluble protein 1:2,000.
* Cases with negative blood culture.

Table I shows the precipitins in the sera of 16 cases of *S. viridans* endocarditis against the bacterial substances. It is evident that the strongest and most uniform reactions are found in the tests with the 2 protein fractions of *S. hemolyticus*, although there are some reactions with the carbohydrate and with the proteins of the other organ-

isms. The weakest reactions occurred in the serum of the only patient in this series in whom clinical history and autopsy findings failed to disclose evidence of rheumatic fever, and in the sera of two patients aged 56 and 69 years. The latter finding may be correlated with a diminishing antibody response with advancing age.[3] Control tests with the sera and the antigens against physiological saline solution were negative. Preliminary heating of 4 of the sera at 60°C. for 30 minutes diminished but did not prevent the formation of the precipitate. In a number of cases weak positive tests were also obtained with a typhoid "nucleoprotein", with which further studies are now being made.

A control group of 100 sera obtained from patients with a wide variety of diseases *other than* rheumatic fever, rheumatoid arthritis, frank hemolytic streptococcus infection, acute nephritis, pneumonia, or peptic ulcer yielded only an occasional positive precipitin test. These reactions in general were much weaker than those observed in the group of *S. viridans* sub-acute endocarditis. Fourteen sera of other patients with temperatures of 102° or more showed no striking precipitin reactions. Three sera from patients with a past history of rheumatic fever and fever due to extraneous causes showed a negative precipitin test.

The relatively marked precipitin formation against *S. hemolyticus* proteins in the sera of the patients with *S. viridans* sub-acute endocarditis is striking and in agreement with Lancefield's findings that an antigenic relationship exists between the nucleoproteins of the *S. hemolyticus, S. viridans,* and *Staph. aureus* organisms. The reaction is not specific for cases of *S. viridans* endocarditis, since precipitates may be obtained against the same antigens with the sera of patients with acute nephritis, rheumatoid arthritis, peptic ulcer, and frequently in the convalescent stage of pneumococcus pneumonia. In a group of cases of nephritis and peptic ulcer, Derick and Fulton[4] obtained a high percentage of positive skin reactions with comparable protein fractions, although their control group showed a much higher number of positive reactions than did the control group of precipitin tests in our series. Coburn has found precipitins against comparable fractions of the hemolytic streptococcus during the active stages of rheumatic fever.[5] In general, however, the tests were weaker than with the sera of the cases of *S. viridans* sub-acute endocarditis.

[3] Thomsen, O., *Z. Immunf.*, 1917, **26**, 213; Thomsen, O., and Kettel, K., *Ibid.*, 1929, **63**, 67.

[4] Derick, C. L., and Fulton, M. M., *J. Clin. Invest.*, 1931, **10**, 121.

[5] Coburn, Alvin F., in press.

The findings in this series suggest that a negative precipitin test with *S. hemolyticus* nucleoproteins as antigens is unusual in cases of *S. viridans* sub-acute endocarditis, except in the aged. Other sera from patients with *S. viridans* sub-acute endocarditis in whom the previous cardiac damage is due to congenital defects, syphilis, or arteriosclerosis are to be studied for precipitins against the bacterial products used in these experiments.

6161

A Laboratory Method for the Diagnosis of Psittacosis in Man.

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Since the pandemic of psittacosis in 1929 and 1930, instances of disease in human beings associated with parrots and parrakeets have continued to appear, either as isolated occurrences or as small localized epidemics. Frequently the patients have manifested unusual clinical pictures and have run courses not considered to be characteristic of psittacosis. Furthermore, at times the disease has occurred in people associated with birds which have apparently been in good health in this country for considerable periods of time and which until recently would have been considered "safe" in the sense of being free from psittacosis.

A reasonably safe laboratory method for the diagnosis of psittacosis in man is of importance. Our investigations, as well as similar experiences of others have indicated that serological tests are probably not suitable for the detection of psittacosis. Following Krumwiede's observations,[1] our work[2] has shown that mice are highly susceptible to psittacosis and that the experimental disease in them can be easily recognized. We have found, furthermore, that this host can be used in a laboratory test for the diagnosis of psittacosis and when infected can be handled with relative safety and with a minimum of danger of accidental infection.

The method now used by us for the laboratory diagnosis of psittacosis in man is briefly as follows: The patient's sputum to which 20-50 volumes of meat infusion broth, pH 7.8, and a small amount of alundun have been added is thoroughly ground in a

[1] Krumwiede, C., McGrath, M., and Oldenbusch, C., *Science*, 1930, **71**, 262.

[2] Rivers, T. M., and Berry, G. P., *J. Exp. Med.*, 1931, **54**, 105.

mortar. The emulsion is centrifuged for 10 minutes at a speed of 3000 R.P.M. Then the supernatant fluid is filtered through a Berkefeld V candle at a pressure of 15-30 cm. of mercury. Each of 6 mice receives intraperitoneally on 3 successive days 2 cc. of the filtrate. The animals are housed in screened battery jars placed in shallow baths of 5% lysol solution in order to prevent the mechanical spread of the infection by insects. All animals are observed for a period of 30 days.

If a patient dies without a diagnosis having been established and if at the autopsy psittacosis is suspected of being the cause of death, confirmatory evidence frequently can be obtained by the injection of filtrates of lung, liver, and spleen into mice in a manner similar to that just described.

The criteria by which the presence of psittacosis in the inoculated mice is established are: (1) the development in some or all of the animals of illness which is usually fatal within 10-14 days, but occasionally not before 30; (2) the characteristic pathological picture[2] consisting of focal necrotic lesions in the liver and spleen; (3) the absence of ordinary bacterial infections as determined from necropsy cultures; (4) the presence in liver and spleen impression smears of the "minute bodies"[3] of psittacosis; (5) the establishment of serial passages of the virus in mice by means of liver and spleen emulsions from the animals receiving the filtrates; (6) the demonstration that mice which have lived for 30 days following the inoculations of filtrates have developed an active immunity against a potent strain of psittacosis virus. All of these conditions obviously need not be fulfilled in each instance, sometimes one, sometimes another serves to establish the diagnosis.

By procedure similar to that described above material from 24 patients in 17 outbreaks or suspected outbreaks of psittacosis has been examined. Of the 24 patients 16 had psittacosis. Unsuccessful attempts were made to demonstrate the virus in 10 of the 16 patients by inoculation of blood into mice. By means of sputum or sputum filtrates, on the other hand, of 11 patients examined, the virus was obtained with certainty from 8, probably from 1, and was not obtained from 1, while 1 examination is still being conducted. In 5 instances the virus was found in the sputum but was not demonstrated in the blood collected simultaneously or earlier in the course of the disease. In 1 case the virus was obtained from sputum collected 48 and 24 hours before death, yet was not demonstrated in the blood or in the organs—brain, lungs, liver, and spleen

[3] Levinthal, W., *Klin. Woch.*, 1930, **9**, 654.

—removed at autopsy. From 1 patient the sputum was collected on the 24th day of illness, a fact that may account for the very small amount of virus present.

From our investigation it is obvious that the sputum is an excellent material in which to demonstrate by mouse inoculation the presence of psittacosis virus, while the blood is not. Bedson's work[4] indicates that the virus can be obtained from the blood of patients provided such blood is injected into parrots or parrakeets. Our endeavors, however, have been directed towards the development of a satisfactory diagnostic test in the mouse, a quite safe host with which to work. It appears that we have been successful, and for more than a year no parrots or parrakeets have been employed for diagnostic purposes in our laboratory.

6162

Normal and Pathological Permeability of the Lymphatic Capillaries in Human Skin.

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It is possible to test the permeability of the lymphatics by means of the vital dyes which have been used to study the permeability of small blood vessels.[1] Observations on the normal and pathological permeability of the lymphatic capillaries in the mouse ear have been reported in previous papers.[2, 3] The present communication concerns itself with the functioning of the lymphatic capillaries in human skin under various conditions. Dyes of graded diffusibility have been used (pontamine blue, Chicago blue, patent blue V, Neptune blue, and phenol red) in the isotonic vehicles 0.9% sodium chloride solution, Tyrode's solution, and a mixture of Tyrode's solution 3 parts and 1 part homologous serum.

Practically any abrasion of the skin, no matter how slight, involves the passage of material into the skin lymphatics. Material thrust into it by scratch, puncture, injection, or superficial cut enters

[4] Bedson, S. P., Western, G. T., and Simpson, S. L., *Lancet*, 1930, **1**, 235, 345.

[1] Rous, P., and Smith, F., *J. Exp. Med.*, 1931, **53**, 219.

[2] McMaster, P. D., and Hudack, S., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 852.

[3] Hudack, S., and McMaster, P. D., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 853.

directly into these vessels. This happens to a much greater degree and much more rapidly than has been assumed. When a drop of isotonic dye solution is placed upon a scarification so mild that no blood is drawn, lymphatic capillaries carrying away the dye from the injured skin become visible in the neighborhood of the lesion almost immediately, under the dissecting microscope. Neutral paraffin oil flooded on the skin enhances visibility. Intradermal injections have been found to be predominantly intra-lymphatic. The results confirm and extend our observations upon the lymphatics of the mouse ear. The more diffusible the dye, the more ready is its secondary escape from the lymphatic channels; and a considerable proportion of the amount of a highly diffusible substance introduced directly into the lymphatics may pass into the interstitial tissue secondarily. Increasing the concentration of the dye enhances passage into the interstitial spaces, but adding serum to the vehicle retards it. Lymphatic capillaries in regions injured by heating, ultra-violet light, or by intradermal injection of bacterial toxins, are far more permeable than usual. In wheals caused by stroking or by histamine the lymphatics are so compromised that they fail to drain away dye stuffs in the usual manner. They are at times closed by pressure and in addition their walls appear to be abnormally permeable. Diffusible dyes devoid of specific affinities for tissue injected directly into acutely inflamed skin escape so rapidly from the lymphatics as to form a very deeply stained spot which contrasts strikingly with the broader and lighter-colored ill-defined patch that forms in a control area as result of the extension of dye within the normal lymphatic meshwork and its relatively slow secondary escape. If the inflammatory process has not progressed to purulence or necrosis dye soon begins to be carried away from the stained spot and decolorization is completed, while much color still remains in the control areas. It is plain that instead of a fixation of material in the inflamed region a more rapid turn-over of it than usual takes place.

It has been generally supposed, on the basis of experiments in anesthetized animals, that the lymph flow from a resting extremity is negligible. Our results show it to be remarkably rapid in the resting arm of normal man. Dye injected in minute amount into the skin of the forearm of a seated subject with the arm resting on a table appears in the large lymphatics of the axilla within 10 minutes. Local injections into normal skin are much less local than has been thought.

6163

Rôle of the NH_2, OH and As = As Groups in Parasitotoxic Action of Arsphenamine Derivatives.

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Some of the derivatives of arsphenamine differ from one another considerably in their *in vitro* toxicity towards trypanosomes but are more alike in their chemotherapeutic activity. The reasons for this are probably manifold. The chemotherapeutically active compounds may be of the RAsO type (Ehrlich,[1] Voegtlin and Smith[2]) and the rates of formation of the substituted arsenious oxides from the RAs = AsR forms may differ, Mayer[3] and Maschmann[4] have suggested that the *in vitro* toxicity is due mainly to the other groups substituted on the benzol ring.

In order to draw conclusions as to the rôle of single groups in the toxicity, we have compared the toxic action of neoarsphenamine on *Trypanosoma equiperdum,* and also the color reaction of the drug with osmic acid, with the same properties of compounds of the RAsO type, lacking one or both of the substituted groups, OH and NH_2 possessed by the arsenious oxide corresponding to arsphenamine.

The reaction of neoarsphenamine with osmic acid (Hiramatsu[5]). To attempt to determine the amount of unchanged neoarsphenamine in a solution of the same exposed to the air, we used Hiramatsu's osmic acid colorimetric method, claimed by the author to determine quantitatively small amounts of neoarsphenamine even in the presence of protein. That a purple color is given by unoxidized solutions of neoarsphenamine or of 3-amino-4-hydroxyphenylarsenious oxide ("Arsenoxide") was confirmed. It was further observed that the reaction can be prevented by the addition of sufficient sodium thioglycollate (a few drops of M/20 $NaOOCCH_2SH$ in about 2 cc. of M/2000 neoarsphenamine or "Arsenoxide"). Although the color is probably due to the formation of colloidal osmium after reduction of osmic acid by the arsenical, the inhibition of reaction by

[1] Ehrlich, P., *Berichte,* 1909, **42**, 17.

[2] Voegtlin, C., and Smith, H. W., *J. Pharmacol.,* 1920, **15**, 475.

[3] Mayer, R. L., *Klin. Woch.,* 1926, **5**, 1699.

[4] Maschmann, E., *Berichte,* 1926, **59**, 1142, 1148.

[5] Hiramatsu, T., *Sei-i-Kwai Med. J.,* 1929, **48**, 133; *Chem. Absts.,* 1930, **24**, 392.

thioglycollate was first interpreted as an action on the —As = As— or the —AsO groups (formation of thioarsenites, Cohen, King and Strangeways[6]). Tests showed that neither p-aminophenylarsenious oxide nor phenylarsenious oxide* give the Hiramatsu reaction, while p-aminophenol *gives* the reaction. Furthermore, the development of color with the latter reagent can also be inhibited by the addition of thioglycollate. Sodium sulfite and sodium thiosulfate inhibited the osmic acid reaction of neoarsphenamine, of "Arsenoxide" and of p-aminophenol.

We conclude that Hiramatsu's test is *not specific* for trivalent arsenic bound to benzol, but rather is due to the joint presence of the amino and hydroxyl groups on the ring (hydroxyaminophenyl grouping) whether arsenic is present or not. Hence the state of oxidation of the arsenic will not be indicated by this test, nor should it be affected by splitting off the arsenic if the hydroxyaminophenyl grouping is unchanged. Since Hiramatsu found the blood of humans to give a positive reaction even several weeks after administration of neoarsphenamine, a considerable part of the injected dose must, at this late time, still bear unchanged $OH.NH_2\phi$ grouping.

Action of quinoid compounds on trypanosomes. Since thiol compounds inhibit the osmic acid reaction of aminophenol (probably an oxidation of the aminophenol) thiol compounds might interfere with the toxicity of aminophenol to trypanosomes, which appears dependent on auto-oxidation of the aminophenol. If commercial preparations of p-aminophenol hydrochloride (as a rule slightly colored) are, after careful neutralization, mixed with trypanosomes suspended in fresh plasma almost immediate parasiticidal action is observed at concentrations above 1%. Purified p-aminophenol, recrystallized as the hydrochloride, is distinctly less toxic to the parasites (Table I). Solutions exposed to the air for several days are more toxic than fresh solutions. Apparently p-aminophenol becomes toxic only as it is oxidized, probably to an iminoquinone. Oxidized p-aminophenol solutions are detoxified by thiol compounds. As we find that p-benzoquinone is highly toxic to trypanosomes, it seems likely that the quinoid configuration is concerned in the toxicity of oxidized aminophenol. The toxic action of quinone is also

[6] Cohen, A., King, H., and Strangeways, W. I., *J. Chem. Soc.*, 1931, 3043, 3236.

* 3-amino-4-hydroxyphenylarsenious oxide was kindly furnished us by Dr. Carl Voegtlin of the National Institute of Health, Washington, D. C., to whom the writers desire to express their gratitude. For the preparation of the other arsenious oxides used in this work, the writers are indebted to Dr. J. S. Buck of these laboratories. Our strain of *Tr. equiperdum* was kindly furnished 3 years ago by Dr. George McCoy, Director of the National Institute of Health.

TABLE 1.
Action of p-aminophenol on trypanosomes and its inhibition by sodium thioglycollate.

No.	Agent	Motility after 15 min. at dilutions* cited: 1/3	1/6	1/12	1/24	1/48	1/96	1/192	Control
1	A = p-aminophenol	—	—	—	—	+	++	++	++
2	A + Na thioglycollate	—	+	++	++	++	++	++	++
3	B = p-aminophenol,recrystallized	—	+	++	++	++	++	++	++
4	B + Na thioglycollate	+	++	++	++	++	++	++	++

Legend: — = not motile, + = slightly motile, ++ = fully motile.

* Technique: Trypanosome emulsion (1) obtained by centrifuging citrated blood of infected rat. p-aminophenol solution (2) = 50 mg. in 3 cc. H_2O, warmed, neutralized with 0.12 cc. 2N NaOH, made to 5 cc. with 0.85% NaCl with which dilutions were also made. Na thioglycollate solution (3) = 0.20 cc. of thioglycollic acid neutralized with 1.60 cc. 2N NaOH, made to 20 cc. with 0.85% NaCl, Exps. 1 and 3: 0.25 cc. of dilutions of (1) +0.25 cc. rat serum + 0.25 cc. of (2) were mixed and motility observed under the microscope, after 15 min. In experiments 2 and 4, 0.25 cc. of (3) were added. Controls the same, without A or B.

TABLE II.
Action of quinone on trypanosomes and its inhibition by sodium thioglycollate.

No.	Agent	Motility at* 5 min.	15 min.	60 min.
5	C = Quinone	—	—	—
6	C + Na thioglycollate	++	++	++

* Technique: Trypanosome emulsion (1) and thioglycollate solution (2) as in Table 1. Quinone solution (3) 20 mg. quinone in 20 cc. 0.85% NaCl solution (4). Locke solution (5) with 0.5% glucose. Exp. 5 = 0.6 cc. (5) + 0.1 cc. (3) + 0.2 cc. (1) + 0.1 cc. (4). After 10 min. 0.2 cc. rabbit plasma. Exp. 6 like Exp. 5 but 0.1 cc. (2) in place of 0.1 cc. (4).

hindered by sodium thioglycollate (Table II). One might think that part or all of the inhibitory effect of thiol compounds on arsphenamine and arsenoxides is due to inhibition of oxidation of the $OH.NH_2\phi$ grouping. But the fact that the toxic action of benzoquinone imine can also be inhibited by thiosulfate and sulfite (Table III), whereas the toxicity of neoarsphenamine is inhibited only by thiol compounds, indicates that part of the toxicity of arsphenamine arises from other than the $OH.NH_2\phi$ grouping. That a part of the toxicity is contributed by this grouping may account for the differences in *in vitro* toxicity between derivatives of arsphenamine with chemically substituted or "covered" OH or NH_2 groups and that of arsphenamine itself in which the groups are free. On the other hand, that *in vivo* many compounds of arsphenamine type behave like the parent substance may be due to ready formation from them all of one and the same "uncovered" arsenious oxide. The positive Hiramatsu test which is due to the presence of $OH.NH_2\phi$ groups, and which is given by neoarsphenamine, supports this view.

TABLE III.

Action of p-aminophenol in the presence of $Na_2S_2O_5$ and $Na_2S_2O_3$.

No.	Agent	Motility after 20 min. at dilutions: 1/8	1/16	1/32	1/64	1/128	1/256	1/512	Control
7	A (Table I)	—	—	—	—	—	++	++	++
8	A + $Na_2S_2O_5$	—	—	+	++	++	++	++	
9	A + $Na_2S_2O_3$*	—	—	+	+	+	+	+	
10	B (Table I)	—	—	—	+	++	++	++	++
11	B + $Na_2S_2O_5$	—	+	++	++	++	++	++	
12	B + $Na_2S_2O_3$	—	+	+	+	+	+	+	

* $Na_2S_2O_3$ solutions are somewhat toxic alone.

Technique: Trypanosome emulsion (1) and solutions (2) of A and B as in Table I. $Na_2S_2O_5$ solution (3) 95 mg. in 10 cc. 0.85% NaCl solution. $Na_2S_2O_3$ solution (4) = N/20.

Experiments: 0.5 cc. of dilution of (2): 0.5 cc. of 0.85% NaCl, or (3) or (4): 0.5 cc. rat citrate plasma (containing about 2 parts of 3.5% sodium citrate solution in 5 parts) + 0.5 cc. (1). Observation after 20 min. Control the same but without A or B.

TABLE IV.

Lack of influence of $Na_2S_2O_5$ on action of 3-amino-4-hydroxyphenylarsenious chloride (D*) on trypanosomes.

No.	Agent	Motility after 20 min. 1/128	1/256	1/512	1/1024	1/2048	1/4096	Control
13	D	—	—	+	++	++	++	++
14	D + $Na_2S_2O_5$	—	—	+	++	++	++	++

Technique: Trypanosome emulsion (1) as in Table I. $Na_2S_2O_5$ (2) solution as in Table III. (3) Solution of D (about M/200) 12.5 mg. in 10 cc. of 0.85% NaCl solution. In Exp. 13, 0.5 cc. of the dilution of (3) + 0.5 cc. of 0.85% NaCl + 0.5 cc. of (1) in rabbit plasma. Exp. 14 the same except 0.5 cc. of (2) replaced the 0.85% NaCl. Control the same without D.

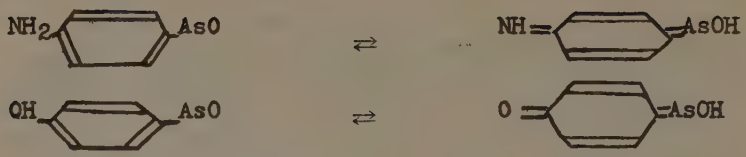
* This substance is assumed to hydrolyze in water giving a hydrate of the organic arsenious oxide.

We next studied the *in vitro* toxic action of arsphenamine derivatives on trypanosomes in the presence of reducing agents and found that they behave distinctly differently from p-aminophenol with respect to detoxification by these agents. Such experiments have been reported by Voegtlin and his coworkers[7, 8] and ourselves,[9] and the following experiments (Table IV) only confirm and complete this picture. Sulfite and thiosulfate were not found noticeably to decrease the toxic action. *In vitro* toxic action was seen even with arsenious oxides not containing both the OH and NH_2 groups. However, it might be assumed that these compounds could also exist in a quinoid modification and thus their activity be connected chiefly with this property. The following tautomerism may exist:

[7] Voegtlin, C., Dyer, H., and Leonard, C. S., *U. S. Public Health Reports*, 1923, **38**, 1882.

[8] Rosenthal, S. M., and Voegtlin, C., *J. Pharmacol.*, 1930, **39**, 347.

[9] Reiner, L., Leonard, C. S., and Chao, S. S., *Arch. intern. pharmacodynamie*, in press.



However, the fact that unsubstituted phenyl arsenious oxide is highly toxic to trypanosomes and that its toxicity is markedly inhibited by thioglycollate proves the essential toxicity of the trivalent arsenic attached to the benzol ring.† It also speaks for the detoxification mechanism by formation of thioarsenites,[6, 8] which we[9] have shown occurs in the medium, shielding the parasites from adsorption of the arsenic. That an additional toxicity due to OH and NH_2 groups may manifest itself when these groups are present is not excluded. Possibly a further contribution by these groups when present "uncovered" may be concerned with a catalysis by the iminoquinonyl-aminohydroxyphenyl oxidation-reduction system of the oxidation of the arsenogrouping to the toxic arsenious oxide stage.

Summary. 1. Hiramatsu's reaction is given by compounds possessing aminophenol groups and the presence of arsenic is not necessary. 2. p-Aminophenol is not toxic to trypanosomes but its autoxidation product, probably iminoquinone, is highly toxic. Reducing agents, such as sulfite, thiosulfate and thiol compounds, inhibit the toxic action. 3. Quinone is toxic to trypanosomes and its toxicity is inhibited by thioglycollate. 4. Various organic arsenious oxides, not capable of giving on oxidation iminoquinone structure, are more toxic to trypanosomes than are oxidized aminophenol and quinone. 5. The toxicity of arsphenamine derivatives to trypanosomes is due chiefly to the presence of trivalent arsenic and not chiefly to hydroxyaminophenyl groupings, although these may contribute to the *in vitro* toxicity.

† Phenyl arsenious oxide is very slightly soluble in water. The test was, therefore, carried out in the following way: 2 mg. were dissolved in 1 cc. of ethyl alcohol and this solution diluted 1/200 with a mixture of 3 parts of physiological salt solution and 1 part of rat plasma. Trypanosomes suspended in dilute rat plasma were killed almost immediately by this solution. Control emulsions containing equal amounts of ethyl alcohol per cc. could be kept for a few hours at room temperature.

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Toxic Action of Hydrogen Peroxide on Trypanosomes and a Note on Chemotherapeutic Mechanism.

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We previously described[1] the toxic action of auto-oxidized aminophenol and of quinone upon trypanosomes and ascribed the action of aminophenols to the formation of quinoid compounds. However, another possible mechanism seemed worthy of consideration. During oxidation processes by molecular oxygen (auto-oxidation) intermediary peroxides are formed which readily hydrolyze in the presence of water forming H_2O_2.* Both the organic peroxides and the hydrogen peroxide may have a toxic action on trypanosomes. The nature of the supposed organic peroxide concerned in aminophenol oxidation is unknown, hence this could not be studied, but it was relatively easy to study the toxic action of H_2O_2 on trypanosomes.

We found that trypanosomes, *Tr. equiperdum,* suspended in Locke solution containing 0.5% glucose were immobilized almost immediately when the added H_2O_2 reached the concentration of 1/30,000. A trypanosome emulsion obtained in the manner outlined in the previous paper was prepared. A solution of hydrogen peroxide was made by diluting 0.1 cc. of Merck's 30% H_2O_2 to 100 cc. with 0.85% NaCl solution. A mixture was made containing 0.6 cc. of the Locke solution, 0.1 cc. of the H_2O_2 solution and 0.1 cc. of 0.85% NaCl solution with 0.2 cc. of the trypanosome emulsion. After 5 minutes no motility was observed. After 10 minutes 0.2 cc. of the fresh rabbit plasma was added and 5 minutes after this slight motility was seen. The addition of catalase or of fresh serum inhibits this toxicity to a certain extent. It was also found that trypanosomes obviously damaged by H_2O_2 will recover after the addition of fresh serum, the catalase content of which decomposes the H_2O_2. Since trypanosomes themselves contain catalase, the action of hydrogen peroxide naturally depends also on the

[1] Reiner, L., and Leonard, C. S., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 946.

* H_2O_2 formation has been demonstrated recently during an auto-oxidation which was catalysed by methylene blue. (Wendel[2].)

[2] Wendel, W. B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 624.

density of trypanosomes present in the emulsion. In this connection it is interesting to note that there exists an optimum density of the emulsion for keeping trypanosomes alive *in vitro* a comparatively long time. The trypanosomes die off quicker in emulsions containing more or less than this optimum number. It is possible that the lower limit of this optimum range is conditioned by the fact that, in the course of the metabolism of the trypanosomes, peroxides are produced to a relatively greater extent if the oxygen content of the milieu is high, *i. e.*, the rate of oxygen consumption is low, the quantity of trypanosomes being relatively small.† Yorke, Murgatroyd and Hawking[3] have pointed out recently that the toxic action of arsenicals is greater in emulsions containing only a few trypanosomes than in dense emulsions. As we have found that only a small percentage of the available arsenical is bound to the trypanosomes, the exhaustion of the arsenical solution by adsorption cannot be the cause of this phenomenon of lessened toxicity when the number of trypanosomes per cc. is great. It seems probable that the strong reducing action of the dense emulsions is concerned in some way with this phenomenon. For example, this might act to free more sulfhydryl groups of the proteins both of the parasites and of their medium. It has been shown that the presence of such groups in the medium acts to shield the parasites by preventing the adsorption of arsenicals.[4, 5]

The formation of H_2O_2 during the auto-oxidation may be considered an accessory mechanism of toxicity of aminophenol solutions and of solutions of arsphenamine derivatives which contain these groups. But one would expect the catalase content of the blood normally to destroy H_2O_2 as quickly as it formed, hence this factor would seem of secondary importance only. It is questionable whether the same can be said of organic peroxides, if these are formed in the course of oxidation of the agent. Heavy metal compounds often poison enzyme action and one might expect arsenicals to exert an inhibitory influence on catalase and hence permit of

† Attempts to demonstrate peroxide formation in trypanosome emulsions have failed so far. This may be because the benzidine test used is not sufficiently sensitive to demonstrate the small amounts which may be present, although they still may damage trypanosomes. 1/30,000 H_2O_2 solutions give a faint benzidine test, yet kill trypanosomes in a few minutes.

[3] Yorke, W., Murgatroyd, F., and Hawking, F., *Ann. Trop. Med. and Parasitol.*, 1931, **25**, 351.

[4] Voegtlin, C., Dyer, H., and Leonard, C. S., *Public Health Rep.*, 1923, **38**, 1882.

[5] Reiner, L., Leonard, C. S., and Chao, S. S., *Arch. Internat. Pharmacodynamie*, in press.

greater H_2O_2 toxicity. Santesson[6] observed that at a concentration of 0.15%, neoarsphenamine faintly speeded catalase action, and below this concentration the arsenical exerted practically no effect. As the concentrations found in the blood stream after injection of chemotherapeutic doses of neoarsphenamine are of the order 0.001-0.01%, the catalase of the host and parasite should function normally to protect the parasites from the H_2O_2 formed in their metabolism or during the auto-oxidation of the arsenical. Santesson[7] proposed that tissue H_2O_2 assists in converting neoarsphenamine into "Arsenoxide".

Conclusion. Hydrogen peroxide is toxic to trypanosomes. The formation of traces of H_2O_2 under conditions where the catalase content of the system is low cannot be excluded. Such concentrations, although not detectable, might be sufficient to exert some damaging action on trypanosomes. While this factor is probably negligible *in vivo*, it still might be involved with the rate of the —AsO formation and thus concerned indirectly with the chemotherapeutic activity of arsenicals (Santesson).

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Effect of Adenine and Caffeine Injections on Behavior of Rats in a Circular Maze.

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Albino rats were trained in a large circular maze until they were able to run from the periphery to the center in the shortest time possible and without making an error. Such trained animals were then given different doses of caffeine and adenine separately and also in combination; the running time, the number of errors, and the general behavior of the rats were noted at various intervals thereafter. Thirty young adult rats, weighing from 100 to 200 gm., were used in these experiments. The dosages varied from 1 to 5 mg. per hundred gm. weight of the animals; the drugs were administered by intraperitoneal injection. In other experiments, the different drugs were given to large rats through a stomach tube; and in

[6] Santesson, S. K., *Arch. f. Physiol.*, 1915, **32**, 405.

[7] Santesson, S. K., *Arch. f. Physiol.*, 1923, **43**, 55.

such cases as much as 10 mg. of these were administered at one time. The number of experiments performed on trained animals with the drugs was 124. Both caffeine and adenine were administered in the form of a weak aqueous solution of the bases (0.2 to 0.4%). As a rule, each rat was given the drug not more than once or, at most, twice a week. An effort was made to determine the effects of adenine and caffeine separately and in combination on each individual animal. The results obtained were as follows:

Caffeine was administered to rats in 51 experiments. In 36 experiments (71%), excitement was produced; in 3 experiments (6%), no effect was observed; and in 12 cases (24%), a depression was noted after administration of the drug. Adenine was given in 40 experiments. A slight excitement was noted in 21%, no effect was observed in 28%, and a distinct depression was produced in 52%. When adenine and caffeine were administered simultaneously in equal doses of from 1 to 3 mg. per 100 gm. weight, the following results were obtained. The total number of experiments was 33; depression was noted in 61%, no effect in 24%, and excitement appearing in 15%.

When adenine and caffeine are administered in combination the effect is an antidynamic one and cannot be explained by a mere summation of the pharmacodynamic action of the 2 components. The excitation produced by caffeine was in most cases weakened or inhibited by the adenine. Tea and coffee affect many individuals in different ways, coffee usually producing greater excitation of the nervous system, as indicated by insomnia, etc. The difference cannot be explained by the difference in quantity of caffeine. Tea differs from coffee especially in containing a large amount of adenine. It is possible that the adenine in tea counteracts the pharmacological action of its caffeine content. This holds good for certain other pharmacological reactions (Macht and Schroeder[1]). The experiments are of further interest in indicating that caffeine, although producing excitation in 70% of the cases, has a depressant instead of a stimulating effect on other animals. This agrees well with the clinical observation that, in some persons, coffee, instead of acting as a stimulant, has a sedative effect and even promotes sleep.

[1] Macht and Schroeder, *Klin. Wochensch.*, 1930, **9**, 2429.

6166

Precipitin Tests as a Basis for a Quantitative Phylogeny.

ALAN BOYDEN. (Introduced by T. C. Nelson.)

From the Zoological Laboratory, Rutgers University, New Brunswick, N. J.

In an earlier report[1] the results of a series of precipitin tests on the sera of certain common Mammalia were given. The degree of reaction as indicated by the titer of the ring tests was expressed as percent of the homologous titers. A new method of using these percent values as a basis for a quantitative phylogeny is here proposed and illustrated.

The method involves the calculation of the average values of the reciprocal relationships between pairs of species. These average values (M) constitute the primary data to be used. The values of M together with their probable errors are given in Table I.

TABLE I.

Average reciprocal values of mammalian sera (M) together with their probable errors, and the values of 100-M for all the species tested reciprocally.

Species	M (%)	$P.E._M$	100-M
Dog vs. Horse	4.9	±1.49	95.1
" " Sheep	5.5	±1.37	94.5
" " Pig	6.2	±0.87	93.8
" " Beef	10.5	±1.5	89.5
Beef vs. Horse	9.4	±1.08	90.6
" " Pig	13.2	±0.78	86.8
" " Sheep	69.3	±4.7	30.7
Sheep vs. Horse	3.7	±0.79	96.3
" " Pig	7.7	±0.92	92.3
Pig vs. Horse	5.5	±0.98	94.5

The least reliable value (dog vs. horse) is still 3.3 times its P.E., and hence the whole series is probably significant. To express these quantitative measures of relationship graphically, it is proposed to use the corresponding 100-M values for the actual distances between the loci of the species. For example, taking dog as the starting point, the distances of the other species from dog are given in the table. The positions of the other species, beef, sheep, pig and horse may then be further defined by their corresponding 100-M values in relation to each other. There will be four 100-M values to be used in establishing the proper locus of each species and all must be so used. The result is shown in Fig. 1, which gives a side view of the spatial arrangement of the loci projected on a plane surface.

[1] Boyden, A. A., *Biol. Bull.*, 1926, **50**, 73.

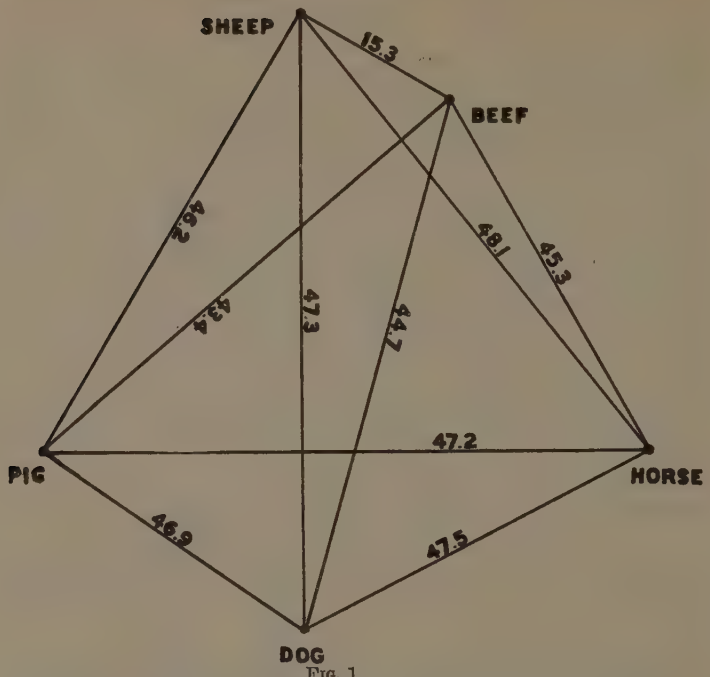

FIG. 1.

The 3-dimensional figure obtained by using the 100-M values of Table I, projected on a plane surface. The unit of distance employed was 0.5 cm., and the numbers shown on each line are the actual centimeter distances of the resulting figure. The figure agrees with the calculated values with an average error per line of 5.8%. None of the lines deviates more than 10.2%.

It should be borne in mind that Fig. 1 shows *only the present relationships* of these 5 species, not their ancestry. The figure does not show time and hence cannot show ancestry. To add the element of time to this geometric figure would require a fourth dimension.

The figure demonstrates 2 significant facts, (1) that the species tested do fall into positions determinable by the primary data, and (2) the present relationships of these species require 3 dimensions for their expression.

Knowledge of phylogeny is of 2 chief kinds: (1) that regarding the present relationships of existing species; (2) that regarding their ancestry. To construct a complete phylogenetic tree requires both kinds of knowledge. Heretofore quantitative measures of present relationships have been almost entirely lacking and information as to ancestry has been even less certain. It may be, how-

ever, that these precipitin tests can give us quantitative measures of the present relationships of existing forms and thus supply exact ideas as to that part of their phylogeny which concerns their present position on the phylogenetic tree. If so the tests will have succeeded in giving us what a century or more of intensive morphological investigation has failed to provide, namely, a basis for a quantitative phylogeny.

6167

Note on the Correlation between Chronaxie and Reaction Time.

R. G. FREEMAN, JR., AND D. WECHSLER.

From the Department of Biological Chemistry, College of Physicians and Surgeons, Columbia University.

The researches of Lapicque and Bourguignon have shown that the excitability of muscles as measured by their chronaxie is closely associated with the form and duration of their contraction, the general rule being that the slower the contraction the longer the chronaxie and vice versa. This has been attested, on the one hand, by the comparatively long chronaxies of smooth as compared with striated musculature (animals) and on the other hand, by the chronaxie changes following nerve degeneration and those met with in the various muscular dystrophies.

In view of this association it seemed to us that there might be some correlation between motor speed and neuromuscular excitability, which might account for differences in speed of movement, on the basis of a constitutional (organic) factor. The present note is a report on the results obtained by simultaneous measurement of the chronaxie and reaction time in 20 normal human subjects.

In obtaining the chronaxie we followed Bourguignon's technique, employing a method described by us in a previous paper,[1] with the apparatus assembled into a simple portable form devised by one of us. The chronaxie was taken over the motor point of the biceps muscle. The average of five readings was taken as the chronaxie.

The speed of bicepital contraction against which we correlated the muscle chronaxie was obtained as follows: Each subject after placing his forearm, fist clenched, upon a table in front of him was

[1] Wechsler, D., and Freeman, R. G., Jr., *Arch. Neur. and Psych.*, 1929, **22**, 558.

instructed, upon a given signal, to move his fist horizontally to the right (a distance of six inches), strike a key and then flex his arm as quickly as possible in a vertical direction. The height and distance through which the subject could flex his arm was limited by an extended board fastened 12 inches above the surface of the table and parallel to it, on the under surface of which was attached a legless telegraph key against which the subject's fist inevitably struck. The interval between the striking of the first and second keys was measured by a 1/100 split second chronometer started by the first blow and stopped by the second, and the interval taken as the reaction time. By breaking up the response into two steps the perceptual component was eliminated.

The table shows the individual data obtained in respect to the 2 variables measured.

TABLE I.

Case	Chronaxie (Σ)	Reaction Time (0.01 sec.)	Case	Chronaxie (Σ)	Reaction Time (0.01 sec.)
1	0.10	1.23	11	0.10	1.15
2	0.10	1.23	12	0.06	1.00
3	0.10	1.47	13	0.10	1.00
4	0.12	1.37	14	0.06	1.10
5	0.06	1.28	15	0.12	1.30
6	0.06	1.01	16	0.06	1.05
7	0.10	1.05	17	0.08	1.10
8	0.10	1.32	18	0.06	1.10
9	0.10	1.20	19	0.06	1.14
10	0.10	1.29	20	0.04	1.00

A correlation, by Sheppard's coefficient method of unlike signs, of 0.84 was found between the two measures. We interpret the results as showing that the speed of muscular contraction, and probably of movement in general is in some way dependent upon the chronaxie of the nerves and muscles involved.

6168

Effect of Antero-Pituitary Hormones upon Blood Sugar.*

JOSEPH EIDELSBERG. (Introduced by H. O. Mosenthal.)

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The reasons which prompted our investigation, and the discussion and possible conclusions, with further data, will be presented at another time. The observations were made on rabbits. The question of whether urinary Hebin (the Antero-Pituitary Sex Hormone obtained from the urine of pregnant women) is identical, derived from or similar to pituitary Hebin (the Antero-Pituitary Sex Hormone obtained from the anterior pituitary lobe), is aside from the purpose of this presentation, but because of the comparative ease with which the former is obtained (as compared to pituitary Hebin) it was used in our work—made from pregnancy urine according to a modification of the Aschheim-Zondek method. Our Growth Hormone was prepared from bovine pituitary by a modification of the Van Dyke method. It was shown to have little luteinizing effect. Both preparations were first tested in animals and showed the characteristic sex and growth responses.

Our observations are then divided into 2 groups: (1) those following injections of Hebin, and (2) those following injections of Growth Hormone. Each of these groups are further considered as (1) acute response and (2) changes after repeated and continued injections (chronic response). All animals were placed on a standard fixed diet—green leaves, lettuce, carrots, and water (later changed to corn, wheat, oats, etc., mixture), and fed once a day. Our blood was taken from a vein in the thigh (1 cc.), and the injections made into the marginal vein of the ear, intraperitoneally, etc. The blood sugar determinations were made according to the Folin-Wu method.

Sex Hormones: Following a control determination, 100 units were injected, and blood drawn at intervals. The rabbits had received no food for 15 to 24 hours. A rise in the blood sugar level occurred, the peak coming from ½ to 2 hours after the injection, the average being about 1 to 1½ hours. We decided to consider a rise of 40 mg. per 100 cc. as the minimum to be interpreted as a

* We are gratefully indebted to Dr. I. Handelman and Mr. S. H. Saffer for their aid in the animal work and care, and the blood sugar determinations, and to Dr. J. A. Morrell, of E. R. Squibb & Sons, for supplying us with the Hormone preparations, and his additional observations and aid.

positive response. Our greatest increase above the control was 222, while most increases were between 100 and 175. We made determinations at various intervals for about 4-5 hours, by which time the blood sugar had fallen again to near or below the original level. A certain (small) number of failures occurred, the percentage awaiting a larger series.

Similar rises were obtained using smaller amounts (25 and 50 units). Injections intraperitoneally likewise gave a rise in blood sugar, varying in amount and in different animals—the rise being delayed 1-2 hours.

In several animals, in whom the above was repeated a number of times (chronic effect), it was noted that the control level became persistently elevated above the original control level (35 to 55).

Growth Hormone: Contrary to the observations indicated above, the injection of Growth Hormone (4 to 6 cc. intravenously and 15 to 30 cc. intraperitoneally) produced but a slight rise or drop in the blood sugar level (20 to 35) in 3 to 5 hours.

Several animals, in whom repeated injections of Growth Hormone were carried out (chronic effect), showed a persistent elevation of the control blood sugar level (30 to 40) above their original control levels.

Conclusions. At this time but little definite conclusion can be drawn. We must allow for normal fluctuations, physiological changes, and the like, due to handling, trauma, and changes resulting from the introduction of foreign materials. Still, certain observations and trends are recorded. The marked hyperglycemia recorded seems unexplainable on the basis of these suggestions.

FOOTNOTE: As to reliability of rabbits for blood sugar studies: (1) Rabbits are used for the standardization of Insulin. (2) Many workers have already done exhaustive studies on rabbit blood sugar levels, and the non-specific expectancies observed as minimal. (E. L. Scott, *et al.*). (3) A large amount of work has been done in determining the hyperglycemic function of Epinephrin, Pituitrin, etc., in rabbits (Nitzescu, Benetato, G. Smith, Watkins, Cori). These men considered rabbits as sufficiently desirable for their work. (4) My own observations in control experiments, led me to set a rise of 40 mg. of sugar per 100 cc. blood as the minimal amount to be interpreted as a positive response.

6169

Absorption of Nitrite After the Oral Ingestion of Bismuth Subnitrate.

ROBERT H. WILSON. (Introduced by Frank P. Underhill.)

From the Department of Pharmacology and Toxicology, Yale University.

Stieglitz[1] advocated the use of bismuth subnitrate in the treatment of hypertension. He suggested that the nitrate was partially reduced in the intestine and that the absorbed nitrite was responsible for the desired therapeutic action. In the majority of cases, nitrite was found in the urine. Ayman[2] recently reported that the drug had no demonstrable effect on the blood pressure. Several cases of nitrite poisoning following ingestion of bismuth subnitrate have been reported.[3, 4] In most cases, large quantities had been given for x-ray examination to patients with open intestinal lesions or hypersensitive mucosae.

If bismuth subnitrate is partially decomposed with absorption of nitrite, the absorption of some bismuth might be expected. The metal has been reported in the tissues and urine following ingestion of the subcarbonate.[5]

It was planned to study the urinary excretion of bismuth, nitrate and nitrite in normal individuals receiving the amount of the subnitrate recommended by Stieglitz. The diet was not rigidly controlled, although variation from day to day was not great. Corned meats and large amounts of vegetables were avoided. 0.5 gm. of the drug was taken 3 times a day for a month. At the end of the period a purge of magnesium citrate cleared the intestinal tract as quickly and thoroughly as possible, and a short after-period was run. Bismuth determinations were made by the method of Leonard,[6] nitrates after Whalen,[7] and qualitative nitrite tests by the Gries (as modified by Hyman and Mann[8]) and Fearon[9] procedures.

Within 2 days of the start of the bismuth subnitrate ingestion, the urinary nitrate nitrogen increased from 4.9 mg. to 57.8 mg. in

[1] Stieglitz, E. J., *J. Am. Med. Assn.*, 1930, **95**, 842; Arterial Hypertension, New York, Paul B. Hoeber, Inc., 1930.

[2] Ayman, D., *J. Am. Med. Assn.*, 1932, **98**, 545.

[3] Beck, E. G., *J. Am. Med. Assn.*, 1909, **52**, 14.

[4] Frick, A., *J. Am. Med. Assn.*, 1924, **82**, 595.

[5] Müller, H., and Kürthy, L., *Biochem. Z.*, 1924, **149**, 239.

[6] Leonard, C. S., *J. Pharmacol. and Exp. Therap.*, 1926, **28**, 81.

[7] Whelan, M., *J. Biol. Chem.*, 1930, **86**, 189.

[8] Hyman, A., and Mann, L. T., *J. Urol.*, 1929, **22**, 521.

[9] Fearon, W. A., *Dublin J. Med. Science*, March, 1920, Fourth Series, No. 1. Abstracted in *J. Am. Med. Assn.*, 1920, **74**, 1128.

24 hours, or over 1000%, and remained at about 800% of the normal during the drug period. Two days after the purge, the nitrate excretion was back to normal.

Bismuth was never detected although the method used measures 0.01 mg. in 100 cc. of urine. All of the samples gave negative nitrite tests with the Gries method, which gives a positive test with less than 0.0001% of nitrite nitrogen. The Fearon test, however, was positive throughout the period of drug ingestion. This was due to the fact that the test is not specific for nitrite. Nitrates, in concentrations such as were found in the urine during the experimental period, will give a green color which more or less resembles the test for nitrites.

A repetition of this experiment with another individual gave similar results. Blood pressure readings on 2 dogs given 2 gm. each of bismuth subnitrate per day over a period of some weeks showed no significant change.

This study neither supports nor opposes the Stieglitz theory. It indicates that the bismuth subnitrate is partially broken down in the intestinal tract, that little, if any, bismuth is absorbed but that the anion is absorbed in appreciable amounts in some form. If the absorption is as nitrite as Stieglitz believes, the ion must be fully oxidized before excretion. The opposite finding of Stieglitz might be explained either as the result of an infection of the urinary tract which would cause a reduction of the nitrate by bacteria in the bladder[8, 10] or from the use of a non-specific test such as we found the Fearon test to be. Stieglitz did not mention the test used by him.

6170

Time of Appearance and Duration of Pregnancy Cell Types in Hypophysis of the Rat.*

H. O. HATERIUS. (Introduced by Eric Ponder.)

From Washington Square College, New York University.

Numerous observers have reported the presence of peculiar cell types ('pregnancy cells') in the pars anterior of the hypophysis during pregnancy. Despite their rather distinctive appearance little is

[10] Salén, E. B., *Acta med. Scandinav.*, 1926, **63**, 369.

* The expenses of this investigation were defrayed by a grant from the Bache Fund of the National Academy of Sciences.

known regarding their allocation with respect to the reproductive cycle. Data are lacking on the time of their appearance following copulation, their persistence, and the effect of parturition and lactation upon their survival.

To determine these points, a large number of carefully selected female rats, with no previous reproductive history, was utilized. The procedure consisted in obtaining material from (1) a series of timed pregnancies, dated from actual copulation or from the discovery of a vaginal plug; (2) a series of *postpartum* animals sacrificed at definite intervals throughout the period of lactation; (3) a series of daily stages following premature withdrawal of litters; and (4) a series of stages throughout pseudopregnancy.

Pituitaries, ovaries, and uteri of all stages were fixed with Zenker-formol-osmic fixative for histological study. Pituitaries were sectioned at 3 microns, other tissues at 5 and 7 microns. The mounted tissues were stained with Delafield's haematoxylin and eosin or with Mallory's triple connective tissue stain.

Time of appearance and duration post coitus. Material from animals of 5 hour, 12 hour, 1 day, and daily stages thereafter throughout the gestational period (22 days) was studied in this series, from 3 to 5 animals being used for each stage in the preliminary work. In the pituitaries of these animals pregnancy cells became discernible on the 3rd day following copulation. The large ovoid cells, with an eccentrically located vesicular nucleus and with a clear, homogeneous cytoplasm, stained deeply with eosin. These cell types were readily apparent by the 4th day. An increase in number and to some degree in size followed until the 12th day, after which time the pregnancy pituitary appeared to undergo no further change. Pregnancy cell types persisted throughout gestation. The pituitaries as a whole appeared to be more loosely organized and more hyperemic than the non-pregnant gland.

Postpartum incidence. In suckling, pregnancy cell types persisted until 3 or 4 days after weaning. This has been tested experimentally, *i. e.,* by premature withdrawal of litters, at the time of parturition and on the 7th, 9th, and 15th days *postpartum;* a normal histological picture was found on the 4th—occasionally on the 3rd—day following removal of the young. In all cases thus far studied the disappearance of pregnancy cell types has coincided with the resumption of the oestrous cycle, as checked by vaginal smears and by histological examination of ovaries and uteri. Pregnancy cells were not found in pituitaries from animals which were in pro-oestrus or oestrus when killed on the 3rd or 4th day after weaning.

Incidence during pseudopregnancy. Data are incomplete and based upon instances wherein copulation did not result in impregnation. A number of pseudopregnancies induced in this manner have been obtained, and a study of cases representing various stages of the pseudopregnant period indicated that pregnancy cells persisted throughout (about 12 days), identical in nature with those occurring during normal pregnancy. They diminished in size, became more granular, and disappeared just before the cycle normally should have re-established itself. However, more cases are necessary to justify definite conclusions on this point.

The fact that pregnancy cell types occur at all during pseudopregnancy would indicate that their presence is independent of embryonic influence. Influence of litter size, moreover, appears to be negligible, since the protocols reveal 2 instances (15 and 17 days *post coitus)* in which only 2 embryos were found *in utero;* nevertheless the pituitaries were characterized by the presence of pregnancy cell types apparently as well defined as in instances of larger litters.

The ovaries of all stages in which pregnancy cells were found were characteristically luteal, and, in view of previous work from this laboratory,[1] it appears probable that the appearance and duration of these cell types are under the influence of the functional corpora lutea of the reproductive cycle.

6171

Calcium, Phosphorus and Cholesterol in Cataractous vs. Apparently Normal Lenses from Human Eyes.*

HELEN UPDEGRAFF. (Introduced by E. P. Joslin.)

From the Chemical Laboratory, New England Deaconess Hospital, and the Massachusetts Eye and Ear Infirmary, Boston.

Lenses used in this study were, for the most part, obtained at cataract operations. A few, however, were obtained at autopsy and in one instance a dislocated lens which was apparently perfectly normal was obtained at operation. Calcium was determined by the method of Fiske and Logan,[1] phosphorus by a modification of the

[1] Haterius, H. O., and Charipper, H. A., *Anat. Rec.*, 1931, **51**, 85.

* This research has been made possible by a grant from Mr. Francis P. Garvan and the Chemical Foundation.

[1] Fiske, C. H., and Logan, M. A., *J. Biol. Chem.*, 1931, **93**, 211.

method of Fiske and Subbarow,[2] and cholesterol by a modification of the method of Bloor, Pelkan and Allen.[3] The weight of 46 lenses studied ranged from 0.1046 to 0.3606 gm. with an average of 0.2237 gm.

Cholesterol determinations on the hot alcohol extract of 42 lenses ranged from 282 to 717 mg. %. Only one fell below 300 mg. % (supposedly normal) and only one above 700 mg. % (a case of immature cataract. All the values are at a much higher level than in normal blood but, with few exceptions, appear to bear no definite relation to age, sex, clinical findings of eye and other conditions, or to the values for calcium and phosphorus. In no case, however, where the lens was supposedly normal (5 cases) did the cholesterol reach a value of 500 mg. %.

Calcium and phosphorus were determined on the residue remaining after 24 hours' continuous extraction of the macerated lens in hot refluxing alcohol.

Values for calcium in 44 lenses ranged from zero in one case of immature cataract to 14,072 mg. % in a case of a dislocated and calcified lens obtained at operation. In 8 lenses supposedly normal, 7 of which were obtained at autopsy and one at operation because it had been dislocated, the calcium ranged from 8.9 to 17.6 mg. %. The level of calcium in the lens would thus appear from this meagre data to be somewhat higher and to have a wider range than in normal blood. Only one lens yielded no calcium and this lens had also the highest cholesterol found so far in any lens (717 mg. %). We were fortunate in obtaining the lens of the other eye (the right eye) in this same patient and the lens showed a low calcium (16.2 mg. %) and a low cholesterol (383 mg. %). Both lenses were diagnosed as immature cataract. Lenses in 2 other cases of immature cataract yielded low figures for calcium (3.6 and 7.5 mg. %) and high figures for cholesterol (572 and 527 mg. % respectively), while one immature cataract had a low calcium (11.3 mg. %) and an average cholesterol (450 mg. %).

One case of mature cataract gave a low figure (7.6 mg. %) for calcium and a high figure (622 mg. %) for cholesterol. Still another, with no diagnosis so far, gave a low figure for calcium (8.4 mg. %) and a high figure for cholesterol (520 mg. %). One case of nuclear cataract had only 3.3 mg. % of calcium and a rather high figure for cholesterol (463 mg. %).

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[2] Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, **66**, 375.

[3] Bloor, W. R., Pelkan, K. F., and Allen, D. M., *J. Biol. Chem.*, 1922, **52**, 91.

In the 28 other cases of cataract studied the calcium ranged from 17.1 (the only one below 20) to 14,072 mg. % in the case of the dislocated and calcified lens already mentioned. There were 3 other very high figures: 61.9 mg. %, diagnosed as immature cataract; 73.4 mg. %, mature cataract and secondary glaucoma; 200.8 mg. %, glaucoma.

In this case of glaucoma we were also fortunate in obtaining the lens of the other eye of the same individual (both were obtained at autopsy). Here the calcium was low, only 11.8 mg. %. The cholesterol in this apparently normal lens was low (374 mg. %) and still lower in the glaucomatous lens (353 mg. %).

Calcium for all the rest of the 28 cataractous lenses was below 56 and above 20 mg. %.

Phosphorus was determined in 39 of the 44 lenses in which calcium was determined. The values for phosphorus in the alcohol extracted material ranged from 3.9 mg. % (a case of diabetes mellitus) to 5,162 mg. % in the case of the dislocated and calcified lens already mentioned, but, as a rule, the values fell between 10 and 30 mg. %.

When the values for phosphorus are considered in conjunction with the values for calcium some interesting possibilities are suggested. Thus in the case of mature cataract and secondary glaucoma mentioned above in which the calcium was 73.4 mg. %, the phosphorus was 38.1 mg. %, practically the exact amount that should be found were all the calcium combined as $Ca_3(PO_4)_2$, the 0.17 mg. % excess phosphorus being well within experimental error. This may, of course, be only a coincidence. It is the only case in which we have found this to be true so far. This and other considerations have led us to undertake a partition of the acid soluble phosphorus and calcium and acid insoluble phosphorus and calcium (if calcium exists in such form) by means of trichloracetic acid. This and other studies are now in progress along the lines indicated.

The writer wishes to gratefully acknowledge the kind cooperation of Dr. Elliott P. Joslin and Dr. J. Herbert Waite in supplying material and facilities for this investigation. Acknowledgment is also due Dr. Y. Subbarow, Dr. M. A. Logan and Miss Hazel M. Hunt for many valuable suggestions.

6172

Production of Thyroid Hyperplasia in Rats and Mice by Administration of Methyl Cyanide.

A. W. SPENCE* AND DAVID MARINE.

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The daily subcutaneous administration of small doses of aliphatic cyanides to young rabbits of either sex maintained on a diet of alfalfa hay and oats has been shown to produce thyroid hyperplasia within 21 days.[1] The most potent cyanide was found to be methyl cyanide. The use of methyl cyanide as a goitrogenic agent has now been extended to rats and mice.

Rats. Twelve female albino rats, 6 being litter mates aged 3 months, and 6 litter mates aged 5 months, were divided into 3 groups, 2 animals from each litter comprising each group. Their diet consisted of hominy 100 parts, rolled oats 25 parts, powdered skim milk 5 parts, dry meat 25 parts, salt 1½ parts and tap water; this was found to be nongoitrogenic. They were given daily subcutaneous injections of varying doses of methyl cyanide in water, the first group receiving 0.08 cc., the second 0.04 cc. and the third 0.02 cc. of methyl cyanide. One animal from each group was sacrificed at the end of 21 days, and all showed only very slight thyroid hyperemia. At the end of 28 days there was definite thyroid hypertrophy, but it was obvious that rats' thyroids were less reactive than rabbits'. During the next 8 days the doses were gradually increased on the remainder in each group, until that on the largest dose was receiving as much as 0.15 cc. of methyl cyanide daily without any clinical sign of cyanide poisoning. After 36 days of treatment the thyroids were larger and more hyperemic. As has been pointed out previously, there were individual variations, but in general the thyroid reactions were proportional to dosage. The results are given in Table I.

Mice. A batch of 12 mice on the same diet as the rats received similar treatment, and were divided into 3 groups, receiving daily 0.005 cc., 0.0025 cc., and 0.00125 cc. of methyl cyanide respectively. Their ages at the beginning of the experiment were 3½ weeks and their weights averaged 13 gm. Only a slight thyroid reaction was produced after periods varying from 11 to 34 days.

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* Fellow of the Rockefeller Foundation.

[1] Marine, D., Baumann, E. J., Spence, A. W., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 772.

TABLE I.

No. of Rat	Age	Wt.	Methyl Cyanide	Duration of Treatment	Condition of Thyroid*
	mo.	gm.	cc.	days	
1	5	230	0.08	28	+ —
			0.1	4	
2	5	260	0.08	28	+ —
			0.1	4	
			0.15	4	
3	3	158	0.08	28	— +
4	3	144	0.08	21	— ?
5	5	202	0.04	33	+
			0.1	3	
6	5	202	0.04	33	+ —
			0.1	3	
7	3	136	0.04	28	— +
8	3	118	0.04	21	— ?
9	5	173	0.02	33	— +
			0.05	3	
10	5	145	0.02	33	— +
			0.05	3	
11	3	114	0.02	16	—
12	3	112	0.02	21	— +

*+ = Twice normal size, moderately hyperemic.
+ — = One and a half times normal size, slightly hyperemic.
— + = Slightly enlarged, slightly hyperemic.
— = Not enlarged, not hyperemic.

Hunt and Seidell[2] demonstrated in "acute" experiments the high tolerance of rats for methyl cyanide, the minimum lethal dose being between 4 and 5 mg. per gm. of body weight. The thyroid reactions obtained in rats and mice by means of the administration of cyanide are far less than those produced in rabbits receiving relatively much smaller doses, and bear out the well-known fact that these animals possess considerable resistance to goitrogenic substances. Since age is such an important factor in both the thyroid response and the animals' resistance to methyl cyanide, it is probable that younger animals would have given better reactions.

[2] Hunt, R., and Seidell, A., Bull. No. 47, Hyg. Lab. U. S. Pub. Health and Marine Hosp. Serv., Washington, 1909.

6173

Effect of Carotene on Course of B. Tuberculosis Infection of Mice Fed on a Vitamin A Deficient Diet.*

M. H. FINKELSTEIN. (Introduced by K. Landsteiner.)

From the Department of Bacteriology, Edinburgh University, Scotland.

The importance of vitamin A as an anti-infective factor in diet has been comparatively well established. Green and Mellanby[1] have shown that rats fed on a diet deficient in vitamin A are very susceptible to pyogenic infection. The addition of carotene raised the resistance of the animals to this infection. While several workers have suspected that vitamin A deficiency plays an important rôle in the resistance of animals to *B. tuberculosis* infection, the exact relationship of this deficiency has not been well established experimentally, although the work of Smith[2] would appear to indicate that it may play some part in lowering resistance to the infection.

The author investigated the effect of addition of carotene to a vitamin A deficient diet used as food for animals infected with *B. tuberculosis*. Mice were selected as experimental animals. Browning[3] showed that histologically, tuberculous lesions in mice closely resemble those found in man and that the disease runs a subacute or chronic course in these animals.

Eighty adult mice from the same stock were fed on a modified Drummond-Watson diet which, with cod liver oil as a source of fat-soluble vitamins, maintained normal growth. The modified Drummond-Watson diet was constituted as follows:

Extracted Casein	1000
Rice Starch	2750
Marmite	250
McCollum's Salt Mixture	250
Cotton Seed Oil	500

Each animal received in addition a daily dose of 0.2 gm. ether extracted wheat germ.

Forty animals were inoculated intraperitoneally with 0.75 mg. of moist 21 days' culture of *B. tuberculosis* (bovine) and on the day of inoculation the animals were divided into 4 groups:

* With the aid of a grant from the British Medical Research Council.

1 Green, H. N., and Mellanby, E., *Brit. J. Exp. Path.*, 1930, **11**, 81.

2 Smith, M. T., *J. Lab. and Clin. Med.*, 1926, **11**, 8.

3 Browning, C. H., *Ed. Med. J.*, 1923.

Group 1	Group 2	Group 3	Group 4
Infected with *B. tuberculosis*	Uninfected	Infected with *B. tuberculosis*	Uninfected

Each group of mice was fed on a fat-soluble vitamin deficient diet. Groups 1 and 2 received a daily dose of 100 antirachitic units of irradiated ergosterol and 0.005 mg. of carotene. Groups 3 and 4 received the same daily dose of irradiated ergosterol as groups 1 and 2 but no carotene. Forty-four days after inoculation all the mice in group 3 had died. During this period the animals in group 4 showed no deaths but many animals showed xerophthalmia. Six deaths occurred in group 1 during the same period. None of the animals in group 2 died nor did they show any signs of vitamin A deficiency. The animals in group 1 all died within 74 days of infection.

In a second experiment 60 adult mice which had previously shown normal growth on Drummond-Watson diet with the addition of cod liver oil, were divided into 3 groups each containing 20 animals and placed on a fat-soluble vitamin deficient diet. Group 1 received a daily dose of 100 antirachitic units of irradiated ergosterol and 0.005 mg. or carotene. Group 2 received the same daily dose of irradiated ergosterol as group 1 but no carotene. Group 3 received no irradiated ergosterol but each animal received 0.005 mg. carotene.

After 60 days, 10 animals from each group were inoculated intraperitoneally with 0.75 mg. moist growth of a 21 days' culture of *B. tuberculosis* (bovine), and the carotene ration of group 3 was increased to 0.01 mg. per animal per day.

The results were as follows:

Group 1. 10 animals Irradiated ergosterol + carotene 0.005 mg.	Group 2. 10 animals Irradiated ergosterol	Group 3. 10 animals Carotene 0.01 mg.
10 animals dead within 11 days.	10 animals dead within 7 days.	10 animals dead within 17 days.

No deaths occurred in the non-infected mice.

All the dead mice in the above experiments were autopsied and cultures made from the spleen, heart blood and gut to detect any possible extraneous infection. No such infection was discovered.

Smears were made from the peritoneal exudate and spleen, and examined for *B. tuberculosis* infection. Numerous tubercle bacilli were found, also polymorphonuclear leucocytes and lymphocytes. Naked eye inspection showed enlarged spleens, tuberculous mesenteric glands, and large tuberculous masses in the lungs.

The above experiments would appear to indicate that *B. tuberculosis* infection in mice deprived of vitamin A and carotene runs a more acute course than in animals receiving 0.005 mg. of carotene per day. There appears to be some evidence that the acuteness of the course of infection varies quantitatively with the amount of carotene added to the vitamin A free diet and that vitamin D is of little importance in determining the course of *B. tuberculosis* infection in adult mice.

6174

Kidney Secretion in Reptiles.

E. K. MARSHALL, JR.

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In birds there is now rather conclusive evidence that tubular secretion plays a major rôle in the excretion of uric acid by the kidney.[1, 2] Uric acid is the main nitrogenous urinary constituent of the arid-living reptiles (snakes and lizards) but no data are available as to its mode of excretion. The experiments reported here indicate that in the lizard, it is chiefly excreted by tubular secretion.

The urine/plasma ratio of uric acid has been compared with that of glucose after administration of phlorizin. Glucose is not secreted by the tubule[3] and phlorizin paralyzes the reabsorptive power of the tubule for glucose. Hence, the urine/plasma ratio for glucose should be a measure of the amount of glomerular filtrate if no glucose is reabsorbed under phlorizin. This error, if present, is probably small and would not affect appreciably the conclusions drawn from the present experiments.

The iguana *(Iguana iguana Shaw)* has been used as the experimental animal. Phlorizin was injected subcutaneously in dosage of 250 mg. per kilo 2 hours before the experiment or in 2 doses of 200 mg. per kilo the afternoon before and early on the morning of the experiment. The iguanas were anesthetized with urethane (5 cc. of a 25% solution per kilo), the cloaca opened with a small incision, cannulae tied into the ureteral papillae, and urine collected for a period of 10 to 30 minutes. The abdominal cavity was then

[1] Mayrs, E. B., *J. Physiol.*, 1924, **58**, 276.
[2] Gibbs, O. S., *Am. J. Physiol.*, 1929, **78**, 87.
[3] Marshall, E. K., *Am. J. Physiol.*, 1930, **94**, 1.

quickly opened and blood drawn from the aorta. The urine which was milky in appearance was diluted with hot water to an appropriate volume to obtain solution of all the uric acid. The plasma was precipitated with tungstic acid. Uric acid was determined by the method of Benedict[4] and glucose by the Hagedorn-Jensen method.[5] The glucose values given were obtained by absorption on yeast. The non-glucose reducing fraction was usually quite large,* but since correction has been made for the diffusion of non-glucose reducing substance into yeast, the results cannot be greatly in error. Neither errors in the glucose values nor errors in the uric acid data (due to the non-specificity of the method) can probably account for more than a small fraction of the differences between the concentration ratios of uric acid and glucose.

Table I gives the essential data of 5 experiments.

TABLE I.
Uric Acid and Glucose in Iguana.

Wt. Kg.	Urine cc. per hr.	Plasma, mg. % glucose	Plasma, mg. % uric	Urine, mg. % glucose	Urine, mg. % uric	U/P Ratio glucose	U/P Ratio uric
1.21	1.20	260	20.8	980	745	3.8	35.8
1.63	2.00	55	22.0	140	1837	2.5	83.6
2.05	0.40	215	24.0	680	1200	3.2	50.0
2.50	1.51	152	15.0	740	313	4.9	20.9
2.50	1.96	276	16.5	420	1110	1.5	66.7

The average concentration ratio for glucose is 3.2 and for uric acid, 51.4. It is evident from these figures that the glomerular filtrate is much too small to account for all the uric acid excreted. Assuming the glucose ratio to be an accurate measure of the amount of glomerular filtrate,† we can calculate that only about 6% of the

[4] Benedict, S. R., *J. Biol. Chem.*, 1922, **51**, 187.

[5] Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, **135**, 46.

* After these experiments were completed, it was found that phlorizin strongly reduces the ferricyanide reagent. Using absorption on yeast to determine the glucose should abolish any error from this source.

† Since these experiments were completed 2 years ago, evidence has been obtained which shows that the glucose ratio after phlorizin accurately measures the glomerular filtrate (Shannon, Jolliffe, and Smith[6]; Clarke and Smith[7]; and Marshall and Grafflin.[8]). It is improbable that phlorizin should cause secretion of uric acid, and in fact the large amounts excreted normally argue against this. In the dog fish Clarke and Smith[7] observed a depression of the secretion of certain substances after phlorizin. If such an effect occurs in the iguana and bird, the proportion of total uric acid secreted would have to be increased.

[6] Jolliffe, N., Shannon, J. A., and Smith, H. W., *Am. J. Physiol.*, 1932, **100**, 301.

[7] Clarke, R. W., and Smith, H. W., *J. Cell. Comp. Physiol.*, 1932, **1**, 131.

[8] Marshall, E. K., and Grafflin, A. L., *J. Cell. Comp. Physiol.*, 1932, **1**, 161

uric acid is eliminated by glomerular filtration and about 94% by some other process—presumably tubular secretion.

It is interesting to compare these experiments on the reptile with similar ones on the bird, where other evidence is available for the secretion of uric acid. In Table II are given results on chickens anesthetized with urethane. Phlorizin was given in about the same dosage as for the reptile, urine was collected from cannulae in the ureters, and blood was drawn at the mid-period of urine collection.

TABLE II.
Uric Acid and Glucose in Chicken.

Wt. Kg.	Urine Flow cc. per hr.	Plasma, mg. % glucose	Plasma, mg. % uric	Urine, mg. % glucose	Urine, mg. % uric	U/P Ratio glucose	U/P Ratio uric
2.5	7.0	381	3.8	6600	604	17.3	159
	8.8	409	4.4	4370	676	10.7	154
1.6	12.0	304	2.9	3700	365	12.2	126
	8.0	302	3.1	4350	572	14.4	185
3.0	14.8	289	7.2	1355	418	4.7	58

The average of the glucose ratio is 12.0 and of the uric acid, 136.4. This means that about 9% of the uric acid is filtered and about 91% secreted, a situation quite similar to that observed in the reptile. The concentration ratio for glucose is much higher in the bird than in the reptile, which can be interpreted as greater reabsorption of water by the tubule (beginning loop of Henle in the bird). In line with this, it may be stated that in neither fish nor amphibian is the glucose ratio greater than that of the reptile, but in the mammal (with complete development of the loop of Henle) the glucose ratio can attain very much higher values than in the bird.

6175

Studies in Renal Denervation (IV).

RUDOLPH HECHT. (Introduced by W. F. Petersen.)

From the Department of Pathology and Bacteriology, University of Illinois College of Medicine.

I. *Distribution of Intra-arterially Injected Oleokoniol.* Rabbits were anesthetized with ether, a lumbar incision made, the viscera pushed to one side, and the aorta isolated and elevated proximal to the renal arteries. The aorta distal to the renal arteries was also isolated and a ligature applied. A hypodermic needle was inserted

in the proximal portion of the aorta and Oleokoniol was slowly injected. The needle was left in place several minutes thereafter to allow for the distribution of the material, and then the proximal portion of the aorta was clamped and the animal killed. The kidneys were removed, sectioned, and stained with Sudan III and hematoxylin. Microscopic examination revealed that the oil was distributed equally on both sides. This procedure was repeated on animals denervated according to the technique of Milles, Müller and Petersen.[1] The left kidney was denervated and the right kidney kept intact for control. The animals were allowed to recover for 2 to 3 weeks before injections were undertaken. Microscopic examination revealed that the denervated kidneys contained more oil than normal kidneys.. This agrees with the findings of Milles, Müller and Petersen,[2] who described the dilatation of the vascular bed in denervated kidneys.

II. *Bacterial Embolism in the Normal and Denervated Kidney.* After having experimented with bacterial suspensions in denervated kidneys,[3] and having determined that the control kidney contained more emboli than the denervated kidney, the effect of chilling and of injection of epinephrine, followed by the intravenous injection of an attenuated *Staphylococcus* suspension was tried.

Rabbits in whom the left kidney had been denervated were used. A 2-week as well as a 2-month recovery period was allowed. The animals were chilled in a bucket of crushed ice and water, until a severe chill had developed. They were then removed from the ice water, and 5 cc. of a concentrated suspension of an attenuated culture of *Staphylococcus aureus* in physiological salt solution was injected into the marginal ear vein of each rabbit. In another group of rabbits, instead of the ice bath, ½ cc. of a solution of epinephrine hydrochloride 1 :1000, was injected subcutaneously, and one minute thereafter 5 cc. of the bacterial suspension was injected intravenously. The animals were killed on the fourth day after inoculation, the kidneys removed, hardened in formalin, sectioned and stained. Microscopic examination again revealed more emboli on the normal than on the denervated side in most cases.

In animals allowed a 2-week recovery period following operation, one "chill" animal and one "adrenalin" animal had approxi-

[1] Milles, G., Müller, E. F., and Petersen, W. F., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 351.

[2] Milles, G., Müller, E. F., and Petersen, W. F., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 354, and 1931, **28**, 561.

[3] Hecht, R., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 212.

mately the same number of emboli in both kidneys. It might be suggested that the shortness of the recovery period may be a factor in this apparent discrepancy. Another explanation is that possibly due to an unavoidable error in surgical technique these kidneys were not completely denervated, and that both sides received the same number of emboli.

The theoretical considerations as to why the normal differs from the denervated kidney in regard to the number of emboli which lodge there has already been discussed.[3]

III. *Microscopic Fat Droplets in the Tubules of the Normal Dog Kidneys.* In our studies of the histology of normal and denervated kidneys, it was noted that dog kidneys always showed fat when stained with Sudan III. It was suggested that fat was possibly only found in the kidneys of old dogs. To prove that microscopic intracellular fat accumulation is or is not a normal constituent of dog's kidneys, the following experiments were undertaken: Several young, normal dogs were bled to death in as short a time as possible. The kidneys were removed, sectioned, and stained with Sudan III. Microscopic examination revealed much fat in the tubular epithelium. The bleeding method of killing the dogs was used to avoid the toxic effect of lethal agents on the kidney epithelium.

To further establish the fact that the fat so demonstrable in the epithelium of the dog's kidney, occurs in dogs with normal renal function, several young, normal, female dogs were taken, day and night urine examined, P. S. P. test, urine analysis, and blood chemistry determinations were performed. All were within normal limits.

These animals were also bled to death, the kidneys removed, sectioned and stained with Sudan III, and again fat was found in the tubules. We, therefore, concluded that microscopically demonstrable fat droplets occur normally in the tubules of dog's kidneys. Experiments to determine the fat content of normal as compared to denervated kidneys are in progress.

6176

Permeability of Normal and Denervated Kidney to Bacteria (V).

G. MILLES AND A. J. NEDZEL. (Introduced by W. F. Petersen.)

From the Department of Pathology and Bacteriology, University of Illinois College of Medicine.

We previously reported the result as indicated by roentgenograms of the injected vascular bed in normal and denervated kidneys following the injection of adrenalin, snake venom and following single and repeated chill.[1]

We here report the results of a series of experiments to determine the permeability of the normal and the denervated kidney to bacteria. Various workers have shown that tubercle bacilli can pass through the anatomically normal human kidney leaving no trace in the organ.[2,3] Petersen and Müller[4] have demonstrated that with the onset the chill in the course of continuous injection of *B. coli* in dogs one of whose kidneys was denervated previously that the organisms can be recovered in the urine from the normal kidney but not from the urine of the denervated kidney. The technic for denervation used is that previously described. After an interval of 2 to 3 weeks following denervation the ureters were incannulated separately and urine collected from each.

In the first series of experiments a suspension of *B. prodigiosus* was injected into the femoral vein and 1 cc. of urine at 5 to 15 minute intervals was plated on plain agar, incubated and the number of colonies counted.

As indicated in Fig. 1, the excretion of organisms from the normal was cyclic reaching 250 per cc. of urine in 15 minutes, 560 per cc. in a half hour, 60 at 45 minutes, 320 at 1 hour, etc., whereas the excretion through the denervated kidney remained at 20 to 60 for the first 1½ hours, rising to 360 at 2 hours and dropping to 50, to rise again at 3½ hours, following in general the output through the normal kidney but at a distinctly lower level.

In the second experiment, Fig. 2, No. I, the same organisms were injected, the urine cultured in the same fashion and 15 minutes after

[1] Milles, G., Müller, E. F., and Petersen, W. F., *Arch. Path.*, 1932, **13**, 233; PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 351, 354, 561.

[2] von Rihmer, B., *Z. f. Urol.*, 1928, **22**, 939.

[3] Wyssokowitsch, W., *Z. f. Hyg.*, 1886, **1**, 1.

[4] Müller, E. F., Petersen, W. F., and Rieder, W., *Verhandl. d. Deutsch. Gesellsch. f. inn. Med.*, 1930, **42**, 580.

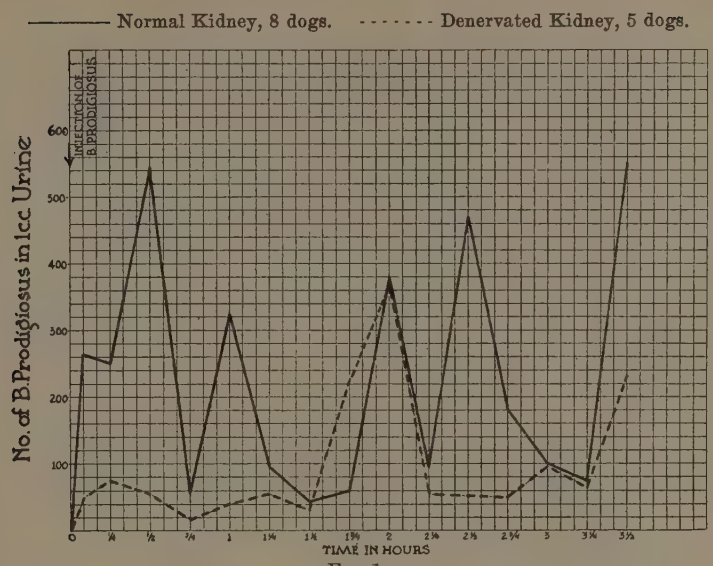


FIG. 1.

the injection of the *B. prodigiosus* 0.5 mg. snake venom per kilo body weight was injected intravenously. The organisms passed through the normal kidney in somewhat smaller numbers, the curve of excretion was prolonged and the drop at the end of 1¼ hours is

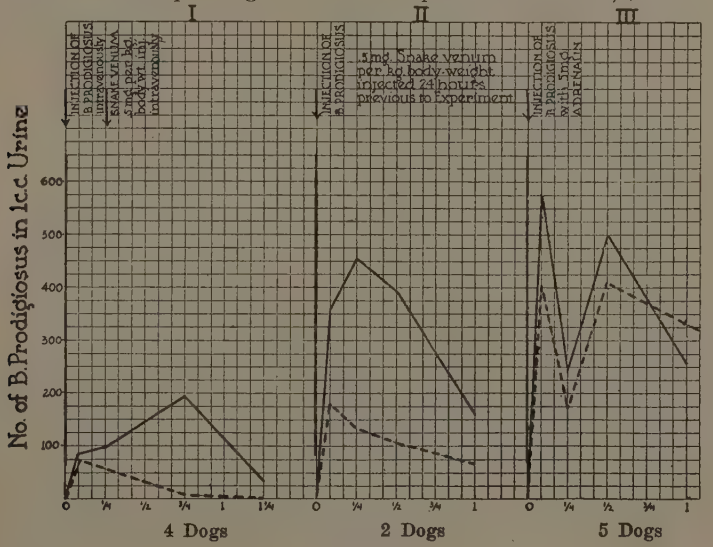


FIG. 2.

more marked than in the same experiment without snake venom. Their passage through the denervated kidney is much less than in the normal animal and again less than through the normal kidney.

In the third experiment, Fig. 2, No. II, 0.5 mg. of snake venom per kilo was injected intravenously and 24 hours later the procedures as outlined in experiment 1 were repeated. The curve of excretion of the organism was shortened as compared to that obtained in 1, the passage through the normal kidney reaching a maximum of 440 organisms per cc. in 15 minutes, dropping to 150 at the end of an hour. The denervated kidney was less permeable to the organisms, their number reaching a maximum of 180 in 5 minutes and falling to 50 at the end of an hour.

In experiment 4, Fig. 2, No. III, the organisms and 5 mg. of adrenalin were injected simultaneously. The curve of excretion was slightly lower for the denervated kidney as compared to the normal kidney, but the difference is by no means as pronounced as in the preceding experiments.

The fluctuation in the excretion curve is much sharper than in the first experiment.

The ability of bacteria to pass through the kidney into the urine indicates that excretion of particles is selective. What is of more importance, the ease with which they pass through the normal kidney as compared to the denervated kidney demonstrates that the state of dilatation or constriction of the renal vascular bed as controlled by the vasomotor nerves (or perhaps by the direct effect of the nerves on the cells) is an important factor in determining the selective excretion of materials by the kidneys.

Considering the selective excretion of bacteria as a dynamic function the effect of a capillary poison such as snake venom in reducing the excretion can be explained on the basis of functional impairment of the cells at the point of excretion, namely, the glomerular tufts, an effect that is accentuated in the denervated kidney, but also noted in the normal kidney.

The effect of adrenalin in enhancing the excretion of bacteria through the denervated kidney, affecting the normal kidney but little, would indicate that the degree of vasoconstriction resulting has little effect since only slight changes are noted in the excretion by the normal kidney but, since the nerve supply is gone, the adrenalin must have a direct effect on the cells involved in the denervated organ.

Summary. 1. Excretion of *B. prodigiosus* by the normal kidney following the intravenous injection of the organisms occurs in more

or less regular waves, a condition whose occurrence in many vital functions has been especially emphasized by Petersen. 2. After denervation the excretion of the organisms by the kidney is greatly reduced and to a large extent the wave-like curve is flattened. 3. The excretion of organisms by the kidney is reduced after the injection of snake venom but their excretion is still much less from the denervated than from the normal kidney. 4. When the organisms are injected with adrenalin their excretion through the denervated kidney is increased, through the normal kidney it remains almost unaffected.

6177

Production of Pylorospasm and Prepyloric Ulcers in Rats.

FREDERICK HOELZEL AND ESTHER DA COSTA.
(Introduced by A. J. Carlson.)

From the Department of Physiology, University of Chicago.

We reported that ulceration of the prostomach of rats following protein restriction was due to the action of the acid gastric juice.[1] An investigation was therefore undertaken to determine whether the gastric acidity was increased by protein restriction. To estimate the acidity, from 15 to 100 pieces of iron, steel or aluminum were given daily to 10 rats, by a method described elsewhere,[2] and the degree of erosion or the percentage of weight lost in passage through the digestive tract was noted. Tests were made for periods of from 47 to over 100 days during which chiefly the protein content of the diets was changed from time to time. Data thus obtained[3] were complicated by factors that tended to obscure evidence of changes in gastric acidity but the net impression was that ulceration of the prostomach occurred as a result of protein restriction without a marked increase in the gastric acidity of any of these rats.

A more definite and striking consequence of protein restriction in 7 of the 10 rats was the development of a gastric retention and a correspondingly increased erosion of test material, in some instances more than 10 times the normal. The retention was appar-

[1] Hoelzel, F., and Da Costa, Esther, PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 382.

[2] Hoelzel, F., *Am. J. Physiol.*, 1930, **92**, 466.

[3] Hoelzel, F., *Science*, 1932, **75**, 311.

ently due to the onset of pylorospasm. As the test material consisted mainly of pieces of iron and aluminum wire, the immediate inference might be that the pieces of metal irritated the pyloric region and spastic contractions were the consequence. But this would not explain why, *in spite of continuing to give the same kinds and amounts of metal daily,* it repeatedly proved possible to clear up growing gastric retentions by simply changing the diet from low to high protein. Moreover, pylorospasm and gastric retention were not observed to be promoted when rats were kept on diets consisting mainly of bran[1] nor when they were given larger amounts of rougher pieces of metal during longer periods in an earlier study but during which only adequate diets were used.[2] On the other hand, chemical irritation or a high gastric acidity seemed to be implicated by the observations that (1) gastric retention generally followed soon after rats were given 25% or more of alcohol and (2) a "smooth" diet of only white bread proved to be particularly effective in producing pylorospasm and gastric retention.

Related to the foregoing was the problem of explaining a seemingly curious variation in the incidence of prepyloric lesions among over 1200 rats that we examined. The lesions were round or oval craters, often multiple and occurring mainly about the lesser curvature in the antrum of the stomach. The depressed centers of the smaller craters frequently showed only moderate changes in the mucosa upon histological examination. The larger craters, however, often had bleeding centers or dark bases and grossly, as well as histologically, appeared as definite ulcers. Prepyloric craters occurred more or less independent of ulceration in the prostomach and they were not always found when gastric retention developed or vice versa. Among 175 control rats without ulcers in the prostomach of any,[1] 3 had multiple shallow prepyloric craters. Among over 1000 other rats in which we attempted to produce lesions, ulcers appeared in the prostomach of about two-thirds and there were 6 perforations, but craters in the pyloric region occurred in only about one-third and no ulcers in this region perforated. Among about 150 rats fed chiefly white bread, over 60% developed prepyloric craters, including some of the largest ulcers found in this region, and about 75% developed ulcers in the prostomach. Fifteen out of 20 rats given either kaolin, barium sulphate or agar with diets adequate in protein developed prepyloric craters but no prostomach ulcers. Thirty-three out of 102 rats fed mainly bran also showed some prepyloric crater formation but, again, without ulceration of the prostomach. The higher incidence and greater devel-

opment of prepyloric lesions on the smoother diets indicates that mechanical irritation can not very well account for them. Our opinion is that the bulky diets gave rise to craters in the pyloric region and prevented ulceration in the prostomach by shifting the acid burden in the stomach toward the pylorus. The opposite took place with starvation, as a result of which the prostomach was always most severely ulcerated. The effect of white bread was probably largely a consequence of the gastric retention which it tends to initiate. In all these cases there seemingly was an excessive exposure of the ulcerated regions to acid gastric juice and often the state of nutrition of the tissues was seriously impaired.

6178

Correlation Between Number of Leukocytes and Percentage of Phagocytosis.

RUTH WESTLUND JUNG. (Introduced by A. A. Day.)

From the Department of Bacteriology, Northwestern University Medical School.

Interpretation of percentages obtained in phagocytic tests on patients undergoing treatment (in this instance, diathermy) is made difficult by the facts that the total leukocyte count in such patients varies and the concentration of leukocytes used in the test sensibly affects the percent of phagocytosis. To evaluate this variation it was necessary to devise a phagocytic test in which the leukocytes would be subjected to the least possible manipulation. Two cc. of heparinized salt solution (containing 1 mg. of heparin per cc. of 0.9% NaCl) are put into a test tube and about 9 cc. of blood added. In a second dry tube about 1 cc. of blood is collected and allowed to clot. The heparinized sample is centrifuged and the cells are washed once with salt solution, then divided into 2 portions. Portion A remains untreated, portion B is deleukocyted by a modification of the method of Fleming.[1] A U-tube is prepared of glass tubing with a constriction in one limb into which absorbent cotton is packed tightly. The other limb is connected to the vacuum apparatus, a little salt solution is drawn through the cotton, and finally the cell suspension B is sucked through 3 or 4 times and is thus rid of most of its leukocytes. By mixing this filtered suspension in various proportions with portion A, a series of blood samples is obtained hav-

[1] Fleming, *Brit. J. Exp. Path.*, 1926, **7**, 281.

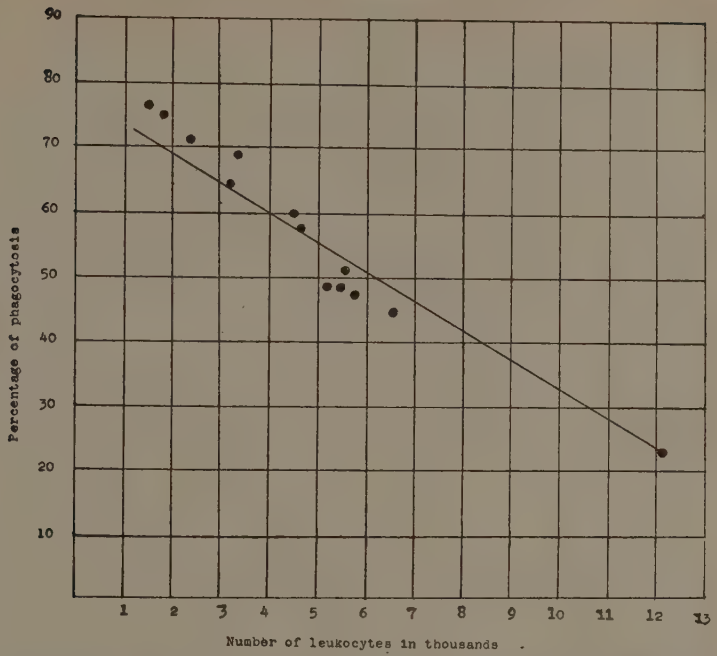
ing the same number of erythrocytes but different numbers of leukocytes.

Phagocytic tests were then run. Two volumes of the blood containing leukocytes are drawn into a bent capillary pipette, followed by one volume each of a suspension of a heat-killed culture of streptococcus and serum from the clotted blood. These substances are mixed by drawing back and forth in the pipette, the end of which is then sealed in the flame and the mixture incubated at 37°C. for 15 minutes. Smears are then made on slides, stained by Wright's method and 50 leukocytes on each edge of the smear counted. The number of leukocytes per 100 containing bacteria is noted and this constitutes the percentage of phagocytosis.

On plotting leukocyte concentrations as abscissae and percent phagocytosis as ordinates, it was clear in every case that the 2 quantities were related. Fifty-six determinations on 10 normal male adults were done on leukocytes collected at one time; a correlation coefficient of —0.83 was obtained. The tests on No. 10 were carried out at varying intervals since the leukocytes from this individual were used as normal leukocytes in opsonic tests on patients undergoing diathermy treatments. The relation appeared to be linear so that for a given subject the data could be represented by a line whose slope m was equal to $\frac{y_2 - y_1}{x_2 - x_1}$ where x = the number of leukocytes and y = the % of phagocytosis. This slope is negative: its mean average for 10 individuals tested was —0.006 and the extremes were —0.012 and —0.002. We thus have a quantity which is independent of the absolute value of the leukocyte count existing at the moment and which can be applied at different times in the same patient. The chart is of a representative case and illustrates the method used in obtaining the slope m.

After setting up the phagocytic tests leukocyte counts are made on the samples of blood used in the test. The ordinary white blood cell counting pipette is used and the number of leukocytes per cmm. obtained. The usual acetic acid solution cannot be used as a diluting fluid. The erythrocytes are not entirely dissolved and appear in clumps holding many of the leukocytes in their midst. The same is true when the blood is collected in an excess of citrate solution. By adding a small amount of $CaCl_2$ this difficulty is obviated. A solution of 1% acetic acid and 0.8% $CaCl_2$ made up in distilled water is found to be satisfactory.

Conclusions. (1) The percentage of phagocytosis obtained in phagocytic tests diminishes as the concentration of leukocytes used is increased. The 2 quantities are inversely proportional. Within the



range from 1500 to 12,000 their relation is practically linear, *i. e.*, represented graphically by a straight line with a negative slope. This slope is a measure of the phagocytic power of the individual's leukocytes. (2) The proportionality constant obtained can be used as a basis for comparing values obtained in the study of phagocytic functions of leukocytes of patients undergoing treatment in which the number of leukocytes is changed.

6179

A Purified Protein Antigen for the Complement Fixation Test in Gonorrheal Infection.*

ARCHIBALD MC NEIL. (Introduced by W. H. Park.)

From the Department of Health Research Laboratories, New York City.

The gonococcus antigen used by Schwartz and McNeil in 1910-11 was a simple suspension of 18 to 24 hours old gonococcus cultures

* I want to thank Dr. Annis Thomson and Miss Pauline Bristol for their assistance and hearty cooperation.

of the 10 strains isolated by Torrey, in 0.85% saline solution, preserved with lysol.

This antigen gave strong cross fixation with anti-meningococcus sera, but did not cross fix with the anti-sera of any of the other pathogens tested. It fixed complement with practically all sera taken from known positive cases of gonorrhea and at the time seemed to give very satisfactory results. In 1912, with Olmstead I succeeded in preparing an aqueous extract antigen filtered through a Berkefeld filter that did not bind complement with antimeningococcus sera, and in experimental work was much more satisfactory than the previous antigens used. When injected intravenously into rabbits it was extremely toxic and produced a serum of high titre. But different preparations varied greatly in their antigenic properties, and while its antigenic value was not impaired by temperatures as high as 80°C. for one-half hour, it was unstable when kept for any length of time and tended to become strongly anti-complementary.

In 1916, working with Wilson, the defatted antigen was developed. This antigen was prepared by treating 18 to 24 hour old cultures of gonococci first with alcohol and then with ether, drying and suspending in 0.9% saline solution. This proved to be a very stable antigen that could be heated to 80°C. without lessening its specificity or antigenic properties. This antigen does not cross fix with anti-meningococcus sera and has been used in routine work from 1916 to the present time with satisfactory results when in the hands of experienced and specially trained technicians.

In April, 1931, a research group was formed consisting of Dr. Emily D. Barringer, Dr. Anna W. Williams, and Dr. Archibald McNeil, to make an intensive study of gonorrhea in women, under a special fund given by Lucius N. Littauer. The cases studied were night court cases sent to the Kingston Avenue Hospital as cases of clinical gonorrhea, and kept there until discharged as cured, making ideal material for the study in question.

Dr. Barringer had complete control of the clinical side of the work, and Dr. Williams the bacteriological and cultural side, and Dr. McNeil the serological work.

The combined results of the 3 studies were correlated with very illuminating results.

It was found that while the complement fixation test with the McNeil-Wilson antigen then in use undoubtedly gave positive results in practically all of the cases of gonorrhea during the period of antibody formation, it left considerable to be desired in regard to

doubtful and weakly positive sera. It was found that if the readings of the tests were made in less than 45 minutes after the antigen controls had cleared, positive readings would be obtained with many sera that should give negative readings, and even with this precaution some sera would continue giving weakly positive readings long after the time when the clinical and bacteriological findings seemed to indicate that a cure had been effected. Cases that had given negative results when the sera was freshly drawn would give indefinite results after the serum had remained for several days in the ice box, and was re-inactivated and re-tested. It was found that after treating the gonococci with alcohol and ether and suspending them in 0.9% saline solution they still maintained their typical morphology and Gram staining properties. The suspension precipitated very rapidly and it was often difficult even with constant shaking to maintain a uniform suspension.

In an attempt to remedy this condition, distilled water was added to the dried gonococci powder resulting from the alcohol and ether treatment, and this suspension placed in the waterbath at 55°C. for half an hour, shaking frequently. This treatment destroys the morphology of the organisms but still leaves the cell protein insoluble. The suspension was then centrifuged at high speed for one half hour, the supernatant fluid decanted and the protein residue suspended in 0.9% saline solution. The supernatant fluid was found to have very little antigenic value and contained lytic and anti-complementary substances. The residual protein suspended in 0.9% saline was found to have lost none of its antigenic strength and to be uniform in its action and to give much cleaner cut results than before the distilled water extraction. The appearance of the suspension was much improved, being much more homogeneous and precipitating very slowly on standing.

But it was still not quite satisfactory as an antigen for routine work. Negative results were still delayed with many known negative sera and apparently cured cases continued to give indefinite reactions. I had previously tried treating the fresh gonococcus cultures with benzine and carbon tetrachloride in place of alcohol, but found them both unsatisfactory. I now tried treating the dried gonococcus powder, that had already been extracted with alcohol and ether, with carbon tetrachloride. The carbon tetrachloride was added to a tube containing the gonococcus powder, and the tube shaken thoroughly. It was then placed in the water bath at 55°C. for 5 minutes, then centrifuged at high speed for 5 minutes. When the tubes were removed from the centrifuge it was found that the pro-

tein matter was floating on the tetrachloride at the top of the tubes while in the bottom of the tube was a considerable amount of crystalline matter.*

The tetrachloride together with the protein was carefully decanted into a filter prepared with a hard filter paper, being careful not to disturb the crystalline matter in the bottom of the tube. The tetrachloride was filtered off and the protein collected and dried on the filter paper. After thoroughly drying the protein on the filter paper, it was weighed and found to have lost about 1% from the tetrachloride treatment. It was then suspended in freshly double distilled water in the proportion of 1.0 gm. to 200.00 C. C. M. and placed in the water bath at 55°C. for 30 minutes, shaking frequently. The suspension was then centrifuged for 30 minutes at high speed, the supernatant distilled water decanted, and the protein sediment suspended in 0.9% saline solution, containing one part in ten thousand of mertheolate as a preservative. The amount of saline added was equal to the distilled water discarded. The distilled water portion was found to be only feebly antigenic, while the protein residue gave a suspension unimpaired in antigenic value and has proven to be specific, and give clear cut positive readings with known positive sera. The negative results were clear cut and most of the readings could be made within 10 minutes after the antigen controls had cleared. Negative sera have been reinactivated and retested day after day with uniformly clear cut negative results and many questionable and indefinite plus-minus results given by the old antigen have been eliminated.

We are now using 5 Torrey strains of gonococci (2 of which are master strains) in the preparation of antigens.

Preparation of antigen for routine work. Five gms. of dried gonococcus powder of each of the 5 Torrey strains, prepared as previously described, is suspended in 1000.00 C. C. M. of freshly double distilled water, this suspension is placed in the water bath at 55°C. for 30 minutes, shaking frequently. The suspension is then centrifuged at high speed for 30 minutes, the supernatant fluid discarded and the remaining protein residue suspended in 1000.00 C. C. M. of 0.9% saline containing one part in ten thousand of mertheolate. This antigen can as a rule be used in a one in 20 dilution in routine work and seems to be free from the undesirable properties of the antigens previously used. One hundred sera of clinical cases have been tested with most satisfactory results. With the

† The amount of crystalline matter varies greatly in different cultures grown on different lots of medium.

antigen as now prepared it should be possible for any technician, who can properly standardize complement and perform satisfactory Wassermann tests, to obtain accurate and reliable results that will satisfy clinicians, with the complement fixation test of gonorrheal infection.

6180

A "Lipoid" Extract of Spleen that Prevents Bartonella Muris Anemia in Splenectomized Albino Rats.

DAVID PERLA AND J. MARMORSTON-GOTTESMAN.

From the Laboratory Division, Montefiore Hospital, New York.

The authors[1] have demonstrated that minute splenic autoplastic transplants made 7 weeks prior to splenectomy protect a large percentage of splenectomized rats from *Bartonella muris* anemia. A comparative histological study of the transplants of protected and unprotected rats revealed a regeneration of the pulp cells in the protected rats and an exhaustion destruction of the pulp in the unprotected rats. This supported the hypothesis that the reticular and endothelial cells of the pulp of the spleen possess some internal secretory substance. The parabiotic experiments of Lauda[2] further support the internal secretory action of the spleen. He found that rats joined by parabiosis are protected if the spleen of only one animal is removed.

Many investigators have tried and failed to demonstrate some substance in the spleen which would replace the spleen in protecting adult splenectomized rats against *Bartonella muris* anemia. We have made many attempts during the past three years to obtain such an extract. Lipoid extracts of the spleen were prepared which possess the property of protecting splenectomized adult albino rats against *Bartonella muris* anemia in a large percentage of instances. Since the anemia in the male is more severe, only male rats were used in testing these extracts. Of 440 male rats of carrier stock used for studies of *Bartonella muris* anemia during the past 3 years, not a single rat failed to develop *Bartonella muris* anemia following splenectomy.

[1] Perla, D., and Marmorston-Gottesman, J., *J. Exp. Med.*, 1930, **52**, 130.

[2] Lauda, E., and Flaum, E., *Z. ges. exp. Med.*, 1930, **73**, 293.

Preparation of the extract. The spleen extract was prepared in a manner similar to Hartman's method of extraction of the suprarenal cortical hormone. Ox spleen freshly obtained from the slaughter house is repeatedly extracted with peroxide-free ether in the dark in an atmosphere of CO_2. The ether extractions are evaporated *in vacuo* at 15-20°. This residue is thoroughly extracted with warm 95% alcohol, then chilled to —10 degrees and filtered. The filtrate is evaporated to one-third the volume and again chilled and filtered and evaporated to dryness. The residue is taken up in a small volume of ether, filtered and dried *in vacuo*. Further extraction with alcohol may be necessary with subsequent chilling and filtering. The final residue is a small amount of thick gummy oil which is difficult to dry. It is thoroughly mixed with the desired quantity of distilled water and filtered through a Zeiss filter, brought up to isotonicity and preserved with 0.1% benzoic acid. One cc. of extract is equivalent to 100 gm. of spleen.

Experimental data. Twenty-nine albino male rats of carrier stock were tested with the lipoid extract of spleen made in this manner. Of these 8 were 6-8 weeks of age and 21 were 3-5 months old. The extract was administered twice daily intraperitoneally in amounts of 0.5 cc. The injections were started 24 hours prior to splenectomy or on the day of operation. The hemoglobin estimation with the Dare hemoglobinometer, the red blood cell count and smears were made daily. It was necessary that protection be observed for a period of one month to eliminate a delayed appearance in the anemia following splenectomy. All the rats were of heavily infected stock raised in our laboratory and used for studies of this anemia during a period of several years.

Of the first group of immature rats complete protection against *Bartonella muris* anemia was obtained in 3 of 8 instances. Of the second group of mature rats 14 of 21 were completely protected. Of 40 male splenectomized rats injected daily with physiological salt solution, all developed *Bartonella muris* anemia.

TABLE I.
Protective action of a "lipoid" extract of spleen against *Bartonella muris* anemia in male splenectomized rats of carrier stock.

No. Rats	Age	Completely Protected	Unprotected	% Protected
Treated				
8	6-8 weeks	3	5	37
21	3-5 months	14	7	66
Controls 0.5 cc. saline twice daily				
40	3-5 months	0	40	0

Rats of carrier stock with the spleen intact, between the ages of 6 and 8 weeks, suffer from a severe infection of *Bartonella muris* with little or no anemia. This is manifested by the occasional appearance of *Bartonella muris* bodies in the blood cells and the marked hyperplasia and congestion of the spleen. Protection in such instances against anemia following splenectomy by an extract of spleen is obviously, therefore less effective. In the adult, the infection is latent and the spleen shows little evidence of hyperplasia and congestion. In these rats protection was obtained in a large percentage of instances.

A "lipoid" extract of the spleen was prepared which protects adult albino rats of carrier stock in a large percentage of instances against *Bartonella muris* anemia following splenectomy. One cc. of extract (corresponding to 100 gm. of fresh spleen) is injected daily intraperitoneally in divided doses for a month. It is suggested that the extract contains a specific hormonal substance.

6181

Protective Action of Copper and Iron against Bartonella Muris Anemia.*

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Steenbock and his coworkers,[1, 2, 3, 4, 5] Beard and Myers[6, 7, 8, 9, 10, 11]

* The copper and iron were obtained through the courtesy of the Myron L. Walker Company, Mt. Vernon, N. Y.

1 Hart, E. B., Steenbock, H., Elvehjem, C. A., and Waddell, J., *J. Biol. Chem.*, 1925, **65**, 67.

2 Hart, E. B., Elvehjem, C. A., Waddell, J., Herrin, R. C., *J. Biol. Chem.*, 1927, **72**, 299.

3 Waddell, J., Elvehjem, C. A., Steenbock, H., and Hart, E. B., *J. Biol. Chem.*, 1928, **77**, 777.

4 Hart, E. B., Steenbock, H., Waddell, J., and Elvehjem, C. A., *J. Biol. Chem.*, 1928, **77**, 797.

5 Elvehjem, C. A., and Kemmerer, A. R., *J. Biol. Chem.*, 1931, **93**, 189.

6 Beard, H. H., and Myers, V. C., *J. Biol. Chem.*, 1931, **94**, 71.

7 Myers, V. C., and Beard, H. H., *J. Biol. Chem.*, 1931, **94**, 89.

8 Beard, H. H., Rafferty, C., and Myers, V. C., *J. Biol. Chem.*, 1931, **94**, 111.

9 Myers, V. C., Beard, H. H., and Barnes, B. O., *J. Biol. Chem.*, 1931, **94**, 117.

10 Beard, H. H., Baker, R. W., and Myers, V. C., *J. Biol. Chem.*, 1931, **94**, 123.

11 Beard, H. H., *J. Biol. Chem.*, 1931, **94**, 135.

and others have established the importance of copper and iron in hemoglobin formation. Young rats fed on a diet of milk deficient in copper develop an anemia within 6 to 8 weeks, that can readily be prevented by the administration of small quantities of copper to the diet. The minimal requirement was 0.025 mg. copper per rat per day. Since albino rats are subject to *Bartonella muris* infection spontaneously during the early weeks following weaning it was thought that the "milk" anemia of the rat may be complicated by infection with *Bartonella muris*. The beneficial prophylactic effect of copper in "milk" anemia suggested its trial in *Bartonella muris* anemia of splenectomized rats.

The rats in these experiments are all carriers of *Bartonella muris* infection. They have been raised in our laboratory under constant environmental and dietary conditions. The diet for the past 10 years has consisted of 15 gm. per rat per day of a mixture composed of hominy 100 parts, rolled oats 25 parts, fine meat and bone 25 parts, dry skim milk 16 parts and salt 1½ parts. Twice a week the rats received whole milk and bread *ad lib.*, and greens (lettuce leaves). The exact quantity of copper in this diet was difficult to estimate but the food mixture was found to contain about 0.025 mg. per 15 gm. of food.† The copper was added in the form of copper sulphate in doses equivalent to 0.1 mg. of elemental copper per day. Lactose was used as a vehicle. The iron was added in the form of iron ammonium citrate. The daily addition of iron was 1 mg. as elemental iron.

Both mature and immature rats were used. The experiments were divided into 2 groups. In one, additions of copper alone, of iron alone and of copper and iron to the basic adequate diet were made during a period of 9-12 days prior to the removal of the spleen, and continued for one month thereafter. In the second group the copper and iron supplements were added to the diet for only 2 days prior to splenectomy and then continued thereafter. Daily hemoglobin estimations and smears were made on all the rats and the red cell counts made every second day.

In these experiments 94 rats were used. The addition of copper or iron to the diet of albino rats in the amounts indicated for 2 days prior to splenectomy and continued after splenectomy, failed to protect in any case adult albino rats from *Bartonella muris* anemia.

Complete protection against *Bartonella muris* infection and anemia, however, was obtained by the addition of copper alone or cop-

† The determinations of the copper content of the diet were made by Dr. Marta Sandberg.

per and iron to an adequate diet when the supplements were given for a period of 10 days prior to splenectomy and continued after splenectomy. Seventy-five percent of 12 adult rats fed copper and 75% of 8 adult rats fed copper and iron were protected. The period of observation is one month following the operation. Copper was definitely more effective than iron, and copper plus iron slightly more effective than copper alone. Fifty percent of 10 rats fed iron were protected. The adult rats were protected in twice as many instances as were the immature rats. This is due to the fact that the severity of the infection with *Bartonella muris* (without anemia) is much greater in the immature rat with intact spleen than in the adult rat in which the infection is entirely latent. Copper protects 75% of the rats against this anemia if it is added as a supplement to an adequate diet for a period of 10 days prior to splenectomy.

These experiments suggest that the utilization of copper and of iron in the body is intimately concerned with the function of the spleen. Copper is involved in some way in the mechanism of resistance in the body as well as in the production of hemoglobin. We have been able to demonstrate an active substance in the spleen[12] which when injected into albino rats from the day of splenectomy protects in a large percentage of instances against *Bartonella muris* anemia. This extract contains neither copper nor iron. The relation of copper to the spleen may be somewhat analogous to the relation of calcium to the parathyroid gland. The small amount of copper in the adequate diet is insufficient for the needs of the rat in the absence of the splenic hormone. An excess of the metallic element may compensate for a deficiency of the hormone. Further, the copper as administered cannot be utilized at once but must be converted into a form that is more readily utilizable by the body in the mechanism of resistance to *Bartonella muris* anemia.

6182

Fever in Certain Cases of Heart Failure.

J. MURRAY STEELE. (Introduced by A. E. Cohn.)

From the Hospital of the Rockefeller Institute for Medical Research, New York.

The great frequency with which fever occurs during the course of heart disease is well known and has been frequently commented

[12] Perla, D., and Marmorston-Gottesman, J., in press.

upon. Its presence has generally been attributed to infection or infarction, usually of the lungs even when no sound proof of their existence was obtainable. In several patients elevations of temperature were so intimately and characteristically associated with the appearance and increase of the signs of heart failure and the return of the temperature to normal so promptly attendant upon the disappearance of these signs, that the processes themselves of heart failure were regarded as responsible for the presence of fever. These phenomena obviously warranted analysis further than the attempt to discover their relation to infection and infarction. The temperatures of patients suffering from heart failure, both during and after recovery, were accordingly studied. The temperatures of the surface (skin) and of the interior (rectum) of the body were recorded simultaneously. Such observations afford a rough estimate of the thermal gradient of the body.

The temperature of the skin being dependent upon environmental as well as upon internal conditions, it was necessary to keep the conditions of the room constant in order to detect changes of temperature in the body. The temperature of the room was maintained constant (21.5 to 22.5°C.). When changes occurred, the temperatures of the skin were corrected according to Vincent's observation; for each degree of change in room temperature, the temperature of the skin was adjusted by 0.3 of a degree.

The temperature, movement of air and humidity were maintained fairly constant. The total effect of these on cooling was measured by recording the cooling power of the air with a Kata-thermometer. This varied between 4.7 and 5.2 millicalories per square centimeter per second for the dry Kata- and 14 and 16 for the wet Kata-thermometer.

The temperatures of the skin were measured with a copper-constantan thermocouple (Benedict, somewhat modified), the rectal ones by ordinary clinical recording thermometers previously compared with the thermometer used for calibrating the thermocouple.

The patient remained undisturbed for 2 hours in a room, the temperature and cooling power of which remained constant, before the first observations were made, and remained there during the whole period of examination. Observations of the temperatures of the skin at 16 points, of the rectal temperature, of the heart rate and pulse rate were usually made every two hours except at 4 a. m. and 6 a. m. The periods of examination varied in length from one to 3 days, before, during or after, the administration of digitalis.

One patient, aged 70, the subject of advanced arteriosclerosis,

enlargement of the heart, rapid auricular fibrillation and heart failure with congestion was relieved by taking digitalis. When digitalis was withheld congestion recurred. Three attacks of heart failure were observed; on each occasion fever was present during the period of failure. No evidence of infection was obtained.

A comparison of skin and rectal temperatures during and after recovery from heart failure showed uniformly that during failure, the temperatures of the skin, more especially those of the extremities, were lower, that of the rectum higher than after recovery. When improvement had taken place, the surface temperatures were closer to each other and closer to the rectal temperatures. The thermal gradient from the interior to the surface of the body was greater during heart failure than after recovery.

This state of affairs is different from that observed in infections in which fever is present in persons whose circulation is supposedly normal for, as is well known, the skin under these conditions suffers an elevation of temperature. The inference which has naturally been drawn is that the elevated rectal temperature in heart failure is due to the difficulties which the embarrassed circulation encounters in distributing properly the heat which is produced within the body.

6183

Relation of Absorption Coefficients to Rate of Penetration of Dye into the Cell.

MARIAN IRWIN.

From the Laboratories of The Rockefeller Institute for Medical Research.

Crystal violet penetrates slowly into the vacuolar sap of *Nitella*. Is this connected with the presence of 0.1 M KCl in the sap? To what extent can such an effect be interpreted by the multiple absorption coefficient theory? This theory deals only with rates and steady states (not with equilibrium); its basic principle is as follows: Other things being equal the rate of penetration of dye into the sap is a function of the concentration gradient (D'_o—D'_s) of the dye in the plasma membrane: D'_o and D'_s represent the concentrations of dye in the plasma membrane at the outer and inner phase boundaries, and are functions of the absorption coefficient* of the dye between the plasma membrane and the aqueous solution at the

* The absorption coefficients may represent solubility or chemical combination or both.

outer and inner phase boundaries. The rate is also a function of the diffusion coefficient of the dye in the plasma membrane.

The following experiments were made to test these conceptions. Employing a cell model[1] consisting of chloroform (representing the plasma membrane) placed between crystal violet (0.04%) at pH 5.5 and artificial sap at pH 5.5 (representing the vacuolar sap) the passage of dye through the chloroform into the sap during one hour was determined colorimetrically. Absorption coefficients were determined by shaking chloroform with the dye solution or with the sap; these are, C_o = conc. of dye in chloroform/conc. of dye in external dye solution, and C_s = conc. of dye in chloroform/conc. of dye in sap.

Results. (1) When $C_o = C_s = 6$, with no KCl in the dye solution or in sap, the rate of penetration of dye into the chloroform and from chloroform into the sap is relatively rapid. The rate is doubled when the concentration of dye in the dye solution is doubled. (2) When $C_o = 6$ and $C_s = 920$, with 0.1 M KCl added to the sap alone, there is no penetration of dye into the sap. The rate of entrance of dye into the chloroform from the dye solution is somewhat less than in (1). (3) When $C_o = 920$ and $C_s = 6$, with 0.1 M KCl added to the external dye solution alone, the dye enters the chloroform and the sap very slightly faster than in (1) but this increase is small as compared with the increase in C_o. (4) When $C_o = 920$ and $C_s = 920$, with 0.1 M KCl added to the dye solution and to the sap, there is no penetration of dye into the sap. The rate of entrance of dye into the chloroform is slightly less than in (3).

These results suggest the following possibilities: (*a*) Addition of 0.1 M KCl to the sap increases the value of C_s, thus decreasing the concentration gradient of the dye in the unstirred layer of the chloroform at the inner phase boundary, which in turn decreases the concentration gradient of the dye in the corresponding layer at the outer phase boundary. *(b)* The rate of diffusion of the dye in the chloroform may be also lowered by KCl owing possibly to the formation of colloidal dye by KCl. (*c*) These 2 changes (*a* and *b*) will reduce the rate of entrance of dye into chloroform from the dye solution as well as that of dye from chloroform into the sap. They will also decrease the effect of the rise in the value of C_o (which would tend to increase the rate by increasing the concentration gradient of the dye in the chloroform).

The slow rate of penetration of crystal violet into the sap of *Nitella* cells may be partly explained on this basis, since the sap con-

[1] For description see Irwin, M., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 135.

tains 0.1 M KCl. But with *Nitella* a slightly soluble dye compound is formed in the sap which would tend to produce a more rapid rate of penetration than would otherwise be the case. Thus if sodium tannate is added to the artificial sap containing 0.1 M KCl, the rate of penetration of dye into the sap is hastened, owing to the formation of slightly soluble dye tannate in the sap.

6184

Cell Models Representing Various Types of Living Cells.

MARIAN IRWIN.

From the Laboratories of The Rockefeller Institute for Medical Research.

The behavior of a variety of cells can be roughly imitated by models[1] consisting of a non-aqueous substance (representing the plasma membrane) placed between dye solution and artificial sap (representing vacuolar sap). The rates of penetration of dye into the sap are compared during one hour. The range of pH values studied is between pH 5 and pH 9.

(1) *When chloroform[1] is used as the membrane.* (*a*) Penetration of dye from cresyl blue solution at pH 9 into the sap at pH 5 has a high temperature coefficient ($Q_{10} = 2.3$ between 15° and 25°C.) This may depend on the change in the viscosity of chloroform. A similar explanation may account for the high temperature coefficient in the penetration of the dye into the sap of *Nitella* and *Valonia.* *(b)* From cresyl blue solution at pH 9 the dye accumulates rapidly in the sap at pH 5 as in *Nitella.*

(2) *When aniline is used as the membrane.* *(a)* The higher the pH value of the cresyl blue solution and lower the pH value of the sap, the more rapid is the rate of penetration and accumulation of dye in the sap. From the solution at pH 9, the dye passes into aniline chiefly as the dye base and upon reaching the sap it is converted to the dye salt. From the solution at pH 5 the dye passes into aniline chiefly as dye salt. (*b*) The lower the pH value of a phenol red solution and higher the pH value of the sap, the greater is the rate of penetration and accumulation of dye in the sap. The dye accumulates rapidly in the sap at a high pH from phenol red solution at low pH; the dye passes into aniline as free acid (yellow) and is converted by the sap to the dye salt (red). This may explain the accumulation of phenol red in the vacuoles of some cells of kidney tubules.

[1] For description see Irwin, M., PROC. SOC. EXP. BIOL. AND MED., 1928, **26**, 135.

(3) When salicylic acid is added to the aniline the rate of penetration of cresyl blue into the sap is so greatly reduced that there is no penetration even from cresyl blue solution at pH 9 into the sap at pH 5.5. But the rate of penetration of phenol red is still rapid from the solution at pH 5 into the sap at pH 8.

(4) When oleic acid is added to chloroform, neither cresyl blue nor phenol red penetrates into the sap at any external or internal pH values.

The rate of penetration of dye into the sap is related to the concentration gradient of the dye in the non-aqueous layer. The rapid rate of penetration and accumulation of these dyes is due to the high concentration gradient: these dyes enter the membrane in undissociated form, and are converted by the sap to the dissociated form, which is not very soluble in the membrane.

Determination of the absorption coefficients shows that the reduction in the rate of penetration of dye into the sap in (3) and (4) is due to the increase in the absorption coefficient of the dye at the inner phase boundary which decreases the concentration gradient of the dye in the non-aqueous layer.

Thus the behavior of various cells toward acid and basic dyes can be very roughly imitated by altering the solutions representing the plasma membrane and the sap.

6185

Bacterial Structure with Particular Reference to the Capsule.

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A technic has been described by which capsules can be readily demonstrated not only on "capsulated" organisms but also on certain bacteria and under certain conditions where they are supposed not to exist.[1] More extensive studies have confirmed the earlier findings, one of the most significant of which was the observation that R types of pneumococcus are as definitely capsulated as S types. Although this method remains the most reliable general capsular stain, with either of the following methods, equally or more beautiful pictures have been obtained, notably with *B. anthracis* from the animal body: modifications of Wright's stain; MacNeal's Tetrachrome Stain; Giemsa Stain; Casares-Gil Flagella Stain. These

[1] Churchman, J. W., and Emelianoff, N. V., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 514.

methods prove that bacterial capsules differ. No one method should be expected to stain all equally satisfactorily. If smears are made from a suspension in 0.25% nutrose, better pictures of the capsule are sometimes obtained.

2. *Structure of Capsule.* The distance between soma and capsular membrane is, like the size of the soma itself, variable. The capsule of virulent pneumococcus enlarges when the organism is injected into the peritoneum of the mouse. The capsule may also shrink and the capsular membrane may then lie practically upon the bacterial body. We have frequently seen 2 distinctly stained capsular membranes, one lying close to the soma and the other at some distance from it, with a large space between. The observations in general suggest that the capsule may in reality be a potential cavity whose size depends on the amount of capsular secretion or other fluid it contains.

3. *Relation of capsule to cortex.* Observations were reported[2] suggesting that many gram positive organisms contain a gram negative medulla and are gram positive only at the surface or cortex. We have recently been able to produce (but not with constancy) bizarre swollen forms of *B. anthracis* in which the medulla (stained pink by Burke's method) can often be seen coursing through the blue black cortex. The relation of capsule to cortex has also now become clear for we have succeeded in staining with differential stains all three layers of *B. anthracis:* the thin pink medullary rod surrounded by the blue black cortex, and quite outside it the eosin stained capsular membrane. Through this capsular membrane chemical interchange must go on freely, for the chemical manoeuvers necessary to bring about partial destruction or decolorization of the cortex show no signs of having affected the capsule in any way.

4. *Relation of capsule to flagella.* If certain motile bacteria are stained by the method of Casares Gil both capsule and flagella are in many instances stained. Usually it is quite clear that flagella arise from the capsular membrane and have no connection whatever with soma. In no instance, when the capsule is stained and the capsular space and membrane accurately located, have we seen the flagella piercing the membrane and passing to the soma. When the flagellum arises from the part of the capsule nearest the observer's eye the appearance may at first suggest origination from soma. In some individuals when the capsular membrane has not taken the stain flagella are clearly seen to originate at some distance from the soma.

[2] Churchman, J. W., *J. Exp. Med.*, 1927, **46**, 1007; *J. Bact.*, 1929, **18**, 413.

Not infrequently when the capsular membrane and space are not demonstrable, the flagella may appear to originate from the somata. Since the capsular membrane may lie practically upon the bacterial bodies and since capsular membrane and soma stain the same color and, if close together, are indistinguishable, it seems not unlikely that in these cases the flagella, though appearing to arise from the bacterial bodies, arise in fact from the capsular membrane itself. The idea that flagella arise from capsule is further borne out by the fact that empty capsules (*i. e.*, devoid of somata) with flagella attached are frequently seen.

5. *Presence of capsule in the acid fast group.* We reported suggestive findings in the study of one member of the acid-fast group *(Mycobacterium tuberculosis bovis)*. The observation was made on specimens stained overnight with carbol fuchsin, decolorized with acid alcohol and then stained by our capsule method. We have since obtained definite evidence of a capsule in this organism by exposing specimens for 3 or 4 minutes to the Casares Gil mordant, washing with water, staining with carbol fuchsin (heated for 3 or 4 minutes, allowed to stand for 10 minutes until cool). The organisms practically always lie in the capsule in an excentric position.

6186

A Quantitative Study of Adrenal Cortical Hormone Extraction.*

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It was demonstrated[1, 2] that cortical hormone can be prepared from whole beef adrenal glands by essentially the same methods of extraction used in the preparation of cortical hormone from dissected cortex.[3, 4] The elaboration of a biological method of assay based on the minimum maintenance requirement of the adrenalectomized dog[5] provided a reliable means of comparing the potency

* This investigation has been aided by a grant from the Josiah Macy, Jr., Foundation of New York.

1 Swingle, W. W., and Pfiffner, J. J., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 510.

2 Swingle, W. W., and Pfiffner, J. J., *Am. J. Physiol.*, 1931, **98**, 144.

3 Pfiffner, J. J., and Swingle, W. W., *Anat. Rec.*, 1929, **44**, 225.

4 Swingle, W. W., and Pfiffner, J. J., *Am. J. Physiol.*, 1931, **96**, 153, 164, 180.

5 Harrop, G. A., Pfiffner, J. J., Weinstein, A., Swingle, W. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 449.

of whole adrenal extract with that prepared from dissected cortex. It has been shown that cortex extract (1 cc. represents 30 gm. beef adrenal cortex) contains 4 to 10 dog units (D. U.) per cc., the potency varying with different batches.[5]

Extracts prepared from whole glands are many times as potent as those prepared from dissected cortex (on an equivalent weight basis). Whole gland extract (1 cc. represents 40 gm. whole beef adrenal gland) contains 40 to 80 D. U. per cc. The following is a summarized comparison of whole gland and dissected cortex extract:

WHOLE GLAND		DISSECTED CORTEX
1000 gm.	=	720 gm. (average)
57.5 mg. active fraction		60 mg. active fraction (average)
25 cc. extract		24 cc. extract
40 to 80 D. U. per cc.		4 to 10 D. U. per cc.
Yield 1000 to 2000 D. U.		Yield 96 to 240 D. U.

Whole gland extracts of approximately equal potency but lower solid content can be prepared by using the same fractionation procedure as previously described but decreasing by 50% the thoroughness of extraction of the respective fractions. In this simplified technique the glands are extracted once with alcohol for 48 hours, the benzene soluble fraction is extracted twice with acetone, the acetone soluble fraction is distributed twice between 70% alcohol and petroleum ether and the alcohol soluble fraction is filtered only once through permutit. The adrenalin concentration is less than 1:2,000,000 (blood pressure-dog). Typical assay data follow:

TABLE I.
Assay on Whole Gland Extract (Half Method).
Ext. HM-2: 1 cc. = 40 gm. gland; 1 cc. = 2.0 mg. solids.
Dog No. 25.

Wgt. kilos	Daily Dose/kg. cc.	mg. solids	gm. gland	Days	Urea N mg./100 cc.	Clinical Condition
12.3	.2	.4	8	7	23-29	Normal
13.3	.1	.2	4	7	28-31	Normal
13.8	.05	.1	2	10	31-34	Normal
14.0	.025	.05	1	7	31-36	Normal
13.5	.0125	.025	0.5	7	31-36*	Variable appetite
12.9	.006	.012	0.24	5	35-90†	Definite insufficiency

Yield per kg. gland = 2000 D. U.

* A dog unit is the minimum daily kg. dose of cortical hormone necessary to maintain normal physiological conditions in the adrenalectomized dog for a period of 7 to 10 days under standard conditions previously described.[5]

† The end-point of the assay consists of a sharp rise in blood urea accompanied or very shortly followed by anorexia, loss in weight, decreased activity and in some cases spasticity of the hind-quarters.

Freezing the whole adrenal glands for 5 months prior to extraction has no effect upon the yield of hormone. A very good yield of hormone was obtained from glands which had autolyzed for 48 hours at room temperature. Extracts preserved with 0.1% benzoic acid at 5°C. retained their activity for 6 months. Longer periods have not been studied. The stability of the finished extract has been checked in two ways (1) adrenalectomized dogs have been kept on a single batch of extract for periods of 3 to 5 months. On withdrawing extract they have come promptly into insufficiency. (2) Assayed extract has been set aside for 6 months and re-assayed without showing any loss in potency.

6187

"Autosterilization" as a Problem in the Bacteriological Examination of Canned Foods.

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During investigations in which large numbers of cans of food spoiled by thermophilic bacteria were opened, many cans with evidences of spoilage contained no living bacteria even though examination of stained films revealed the presence of many cells. This seemed to indicate that the microorganisms responsible for the spoilage had probably died out. To secure more information on this situation which has become known as "autosterilization", the problem was attacked with canned corn and several spoilage bacteria including an active thermophilic spoilage organism, No. 1518, in both tin and large culture tubes.

Large culture tubes were half-filled with fresh canned corn, layered with paraffin and sterilized in the autoclave. The corn in the tubes was then inoculated with the spoilage organisms and divided into 4 sets. One set was incubated at 10°C.; the second at room temperature (20-37°C.); the third at 37.6°C., and the fourth at 55°C. At intervals of 2 or 3 days a tube from each group was removed, plated for the number of viable organisms, and the pH determined. A relationship between the temperatures at which the tubes were held and the number of living organisms was apparent with all cultures of thermophilic bacteria used. At 55°C. a lethal H ion concentration was reached, much more quickly than at 37°C.

The tubes which were held at 55°C. had an original pH of 6.8 and a bacterial count of 4,000,000 per gm. At the end of 15 days, the count was reduced to 100 per gm. with a pH of 4.8. Further incubation at the same temperature for 4 or more days killed all of the organisms with practically no change in pH. A similar experiment was carried out with No. 2 cans of corn. These cans were proven to be free from viable spoilage organisms before their use. They were then opened aseptically and inoculated with spores of organism No. 1518. Before inoculation, the cans were thoroughly heated in the Arnold and a small opening made, through which the spore suspension was introduced. There were 3,000,000 viable spores per cubic centimeter in the suspension. The cans were then incubated at 55°C., one can being opened each week over a considerable period of time. No viable organisms could be demonstrated in the contents of the cans after 28 days, when the hydrogen ion concentration had reached 4.8. The rest of the cans in the series were then opened and similar results secured. It was found that the reaction of the inoculated incubated corn became acid very rapidly, and reached its maximum at the end of 2 weeks. In another experiment, viable cells were absent after 9 days. The pH was as reported above.

A few experiments with an organism producing putrefactive spoilage of corn did not indicate that autosterilization occurred. The optimum temperature of this organism was 37°C.

6188

Penetration of Intestinal Wall of Rabbit by Yeasts.

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Casagrandi[1] early concluded that blastomycetes seldom penetrate the mucous layers of the intestinal wall. Positive results were explained on the basis of existing injuries which escaped detection. Fisher,[2] however, recently found that yeast fed fasting dogs is absorbed like bacteria into the portal circulation. Cells injected into the portal vein also proved that yeasts can invade all the organs, the distribution varying with the amount injected.

[1] Casagrandi, O., *Annali d'Igiene sperimentale*, 8. Reviewed in *Cent. Bakt.*, 1908, **1**, Ref. 24, 758.

[2] Fisher, Virginia, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 948.

In this study the blood of 30 rabbits, daily fed large doses of viable yeast cells, was frequently cultured. The heart's blood, bile, the spinal cord, and portions of certain of the vital organs including the liver, lungs, spleen and kidneys were cultured upon autopsy. Blood sera of the animals were also periodically examined for the presence of agglutinins.

Before feeding the blood of experimental and control animals was cultured and found to be free from yeasts. The yeasts isolated from the blood and organ cultures were compared with the strain which each animal received.

The following varieties of yeast were used: *Saccharomyces carlsbergensis, Saccharomyces cerevisiae, Saccharomyces glutinis, Saccharomyces turbidans, Endomyces albicans, Monilia tropicalis, Torula cremoris,* Hunter's yeast isolated from oysters, a commercial pressed yeast, *Saccharomyces ellipsoidens, Willia saturnus, Zygosaccharomyces mandshuicus, Monilia albicans, Monilia candida, Mycoderma cerevisiae, Oidium albicans, Torula hansen, Torulopsis sanguinia,* and yeast isolated from a sore throat. The first 9 species listed were recovered from the blood after feeding. All, with the exception of a commercial pressed yeast were taken from stock cultures in the laboratory. Suspensions of the commercial yeast were prepared by dissolving a 15 gm. cake in 50 cc. saline, 4 to 6 cc. of which were given certain of the rabbits daily. The remaining animals were fed saline suspensions of yeasts grown on dextrose agar slants at room temperature. No attempt was made to feed exactly the same number of cells or cells of the same age each day, the primary purpose being to keep the alimentary tract of each animal heavily seeded with yeasts.

The animals were bled regularly from the marginal vein of the ear. Bleeding from the ear was found to be much more satisfactory than from the heart.

Macroscopic agglutinations were made with sera dilutions of 1-10, 1-20, 1-40, 1-80, 1-160, 1-320, and 1-640. Suspensions of the cells of 48 hr. cultures (room temperature) in 10 cc. of saline were used as antigen. The tubes were observed frequently after being held an hour at 37°C. and were reported as positive when showing clumps and flakes suspended in the serum and saline. It was found that if all tubes were completely inverted before reading positive results were more easily detected. The precipitate in the control would spread uniformly through the tube while the clumps in positive ones would remain suspended in the liquid. Seven out of 21 animals showed the presence of agglutinins, at the end of

the first month, 8 out of 17 at the end of the second month, and 11 out of 16 fed for 3 months. Maximum agglutination (1-320) was ordinarily demonstrated within 3 or 4 hours although reactions could be read when tubes were held overnight.

Portions of the serum obtained before and during feeding were inoculated with the various yeasts fed and held at body and at room temperatures. In this study blood serum was never found to exert noticeable effect upon the yeast fed when held at room temperature. Some yeasts, however, failed to develop as well as others upon transplanting to dextrose agar slants after being held in fresh blood serum at 37°C. for a period of 48 hours. That was not due entirely to any direct action of the serum itself, nevertheless, as the same yeasts also failed to develop readily when inoculated on dextrose agar slants and held at that temperature.

6189

The pH and the CO_2 Content of Cerebrospinal Fluid in Epilepsy.*

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Although considerable work has been done on the acid-base relationships in epilepsy, the evidence presented thus far has been conflicting and indecisive. Because of the importance of this phase of the problem, we have determined the pH and CO_2 content of the cerebrospinal fluid in 125 epileptics and 30 non-epileptic control subjects by procedures which eliminate errors due to loss of CO_2.[1, 2]

The colorimetric method of McQuarrie and Shohl[1] used for determining the pH may be briefly described as follows: The clear fluid is drawn into a special glass sampling burette over clean mercury after all air in the connecting tubes has been eliminated by means of a 3-way stop-cock. In preparation for taking the sample a number of tenths of a cc. of 0.0075% phenol red equal to the number of cc. of fluid to be taken is measured into the appara-

* The writers are grateful to Dr. W. T. Shanahan, Superintendent of Craig Colony, for his cooperation during these studies, and to Dr. Edith Boyd for assistance in the use of the statistical method.

[1] McQuarrie, Irvine, and Shohl, A. T., *J. Biol. Chem.*, 1925, **66**, 367.

[2] Van Slyke, D. D., and Neill, J. M., *J. Biol. Chem.*, 1924, **61**, 523.

tus. After the spinal fluid is mixed with the dye in this concentration, it is read directly at 38°C. against the bicolor standards of Hastings and Sendroy in a simple comparator block made to hold the sampling burette and standard tubes which have the same diameter. By slightly elevating the mercury reservoir of the sampling tube and turning the stop-cock of the latter, spinal fluid is forced into an Oswald-Van Slyke pipette. This is then measured directly into the Van Slyke and Neil[2] apparatus under mineral oil for determination of the CO_2. In the calculation of the CO_2 allowance is made for the dilution of the spinal fluid by the dye solution.

Several facts suggest that the occurrence of epileptic attacks depends at least in part upon a disturbance in the mechanisms regulating acid-base equilibrium. Certain procedures, which are often effective in preventing seizures, such as fasting,[3] the use of strongly ketogenic diets,[4, 5] and the administration of acid-forming salts or carbon dioxide,[6] tend to produce mild acidosis. On the other hand, alkalosis from hyperventilation of the lungs,[7] from administration of alkali[6, 8] or even the use of alkaline ash diets[8] favors the occurrence of seizures. Although a number of workers[9, 10] have reported evidence of an instability of acid-base balance in the blood in relation to *grand mal* attacks, others[11] have been unable to confirm this. The spinal fluid has been studied very little from this angle. Patterson and Levi,[12] using an "open" colorimetric method, found an average pH value of 7.75 in 50 cases of epilepsy. Osnato and Killian[13] found normal values in a small series of cases by use of the "closed" method of McQuarrie and Shohl.[1] Since Gesell and Hertzman[14] had shown that the pH of the cerebrospinal fluid may differ considerably from that of the blood under certain circumstances, it was thought desirable to restudy the spinal fluid in a large series of cases under uniform conditions and by means of a simple but accurate technique.

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[3] Geyelin, H. R., *Med. Rec. N. Y.*, 1921, **99**, 1037.

[4] Wilder, R. M., *Mayo Clinic Bull.*, 1921, **2**, 307.

[5] Peterman, M. G., *Am. J. Dis. Child.*, 1924, **28**, 28.

[6] Lennox, W. G., and Cobb, Stanley, *Medicine*, 1928, **7**, 105.

[7] Foerster, O., *Deutsche Z., f. Nervenh.*, 1924, **83**, 347.

[8] McQuarrie, Irvine, and Keith, H. M., *Am. J. Dis. Child.*, 1929, **37**, 261.

[9] Jarlov, E., *Compt. rend. Soc. de biol.*, 1921, **84**, 156.

[10] Geyelin, H. R., Bigwood, E. J., and Wheatley, M. A., PROC. SOC. EXP. BIOL. AND MED., 1923, **21**, 227.

[11] Marrack, J., and Thacker, G., *Brit. J. Exp. Path.*, 1926, **7**, 265.

[12] Patterson, H. A., and Levi, P., *Arch. Neurol. and Psychiat.*, 1926, **15**, 353.

[13] Osnato, M., and Killian, J. A., *Brain*, 1927, **50**, 581.

[14] Gesell, R., and Hertzman, A. B., *Am. J. Physiol.*, 1926, **78**, 610.

Most of the epileptics examined were mildly to moderately severe institutional cases of various types, ranging in age from 6 to 18 years. The non-epileptic control subjects cannot be said to have been "normal" persons, but none of them showed signs of acute illness or other conditions likely to be associated with an abnormal acid-base balance. The group was constituted as follows: Mongolian idiocy 3, congenital hydrocephalus 3, chorea minor 2, brain tumor 3, "behavior problem" cases 5, post-encephalitic syndrome 2, congenital syphilis 4, feeble mindedness 5, post-poliomyelitic paral-

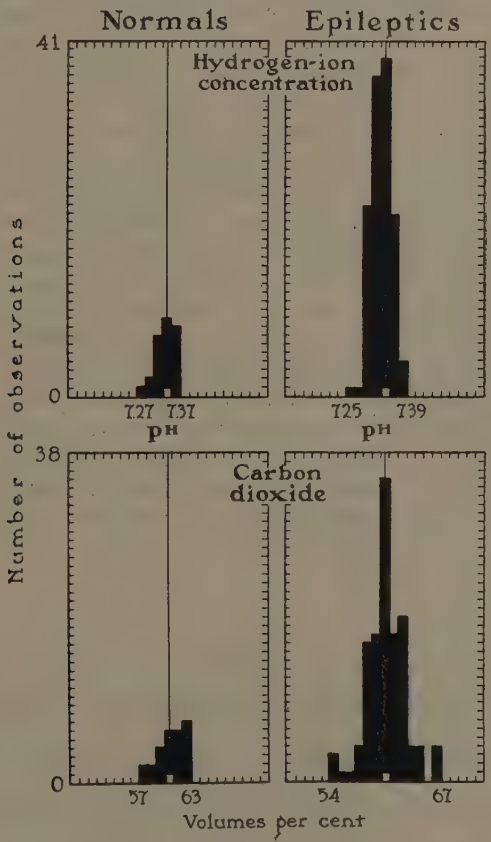

FIG. 1.

Histograms for the pH and CO_2 content of the cerebrospinal fluid in non-epileptic and epileptic subjects.

The white square indicates the mean of the frequency distribution for each constituent. The perpendicular solid line indicates the means for non-epileptic subjects.

ysis 3. All samples were taken in the morning before breakfast. Every subject examined had previously been on an ordinary mixed diet and approximately one-third of the epileptics were on phenobarbital therapy. Samples were obtained in several instances within one hour after a seizure. The shortest time interval between the lumbar puncture and a subsequent *grand mal* attack was 1.2 hours.

Figure 1 gives the results. The mean values for the pH (7.327) and the CO_2 content (60.0 vol. %) of the epileptic patients are practically identical with those found for the non-epileptic control group (pH 7.328, CO_2 60.6 vol. %). In a few non-epileptic cases in which the comparison was made, fluid taken from the lateral ventricles did not differ significantly, as regards these factors, from that obtained simultaneously by lumbar puncture. In but one epileptic and one non-epileptic was the pH reading below 7.30. This could readily be accounted for in the case of the former as a result of a severe convulsion which occurred a few minutes before the sample was taken. The pH was 7.25 and the CO_2 content 66.5 volumes percent, showing the low pH to be due in part to an accumulation of CO_2. The only low pH value (7.28) for the control group was found in the case of a mongolian idiot, who struggled violently before the lumbar puncture could be made. The maximum pH for the epileptics was 7.39, which occurred 4 times only and that for the non-epileptics 7.37, a difference well within the range of error in measurement. No values were found which would indicate the slightest tendency toward an "alkaline drift" in the spinal fluid of the epileptics. In contrast with the findings of Geyelin, Bigwood and Wheatley[10] for blood, the range of variation in pH values for spinal fluid was remarkably narrow in our series.

6190

The Electrocardiogram in Coronary Thrombosis.

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The tentative conclusions below are based upon a study of the electrocardiograms in 56 cases of coronary thrombosis, in 17 of which a post-mortem examination of the heart was made.

The electrocardiogram is of great value in the diagnosis of this condition, particularly when it is possible to obtain a series of curves.

The most important feature of the abnormal *T*-deflections (described by Smith,[1] Pardee[2] and others) that occur in cardiac infarction is the characteristic and progressive change in form which they undergo. These changes in the *T*-deflection are usually accompanied by changes in the form of the initial deflections *(QRS)* which have not been fully described although the large *Q*-waves that frequently occur in lead III have received considerable attention (W. J. Wilson,[3] Parkinson and Bedford,[4] Levine and Brown,[5] Pardee[6]). The curves may be divided into 2 groups:

(a) In typical curves of the first group the initial ventricular deflections of lead I are usually of small or medium amplitude, and there is a broad and conspicuous *Q*-deflection in this lead. In leads II and III the first initial deflection is upward and is followed by an S-wave, often of large amplitude. These changes in *QRS* occur early and commonly outlast the *T*-wave changes that accompany them. In the early stages of cardiac infarction there is, in curves belonging to this group, an elevation of the *S-T* segment in lead I and a depression of this segment in lead III. This partial fusion of *R* and *T* in the first lead and of *S* and *T* in the third often gives rise to curves which resemble in general outline the monophasic responses yielded by injured heart muscle, as Clarke and Smith[7] have pointed out. In the later stages there is a progressive inversion of *T* of lead I; the final portion of *T* is the first to become inverted and the inversion progresses backward toward *QRS* so that *T* finally becomes a deep V-shaped depression. Changes of the opposite type occur in lead III in which *T* is converted into a deflection which resembles an inverted V in form. In a small number of cases in which the standard leads yielded curves of this type serial precordial leads[8] were also employed. The electrocardiograms so obtained are unlike those that we have obtained by a similar method of leading in any other condition. The chief initial deflection of these curves, which is upward and of large amplitude, is not preceded by a downward deflection as is ordinarily the case. The *T*-deflection of these special

1 Smith, F. M., *Arch. Int. Med.*, 1918, **22**, 8.
2 Pardee, H. E. B., *Arch. Int. Med.*, 1920, **26**, 244.
3 Wilson, W. J., *Ann. Clin. Med.*, 1926-27, **5**, 238.
4 Parkinson, John, and Bedford, D. Evan, *Heart*, 1927-29, **14**, 195.
5 Levine, S. A., and Brown, C. L., *Medicine*, 1929, **8**, 245.
6 Pardee, H. E. B., *Arch. Int. Med.*, 1930, **46**, 470.
7 Clarke, N. E., and Smith, F. J., *J. Lab. and Clin. Med.*, 1926, **11**, 1071.
8 Wilson, F. N., Macleod, A. G., and Barker, P. S., *Am. Heart J.*, 1932, **7**, 305.

leads is strongly positive when T of standard lead I is sharply negative. When T of lead I is not inverted, however, the T-deflections of precordial leads may be negative.

(b) In typical curves of the second group large Q-waves occur in lead III and usually in lead II as well; in lead III Q is often the largest of the initial deflections. As a rule no Q wave is present in lead I. The T-wave changes that occur in lead I are similar to those that occur in lead III in the first group and vice versa. In the late stages of cardiac thrombosis T is sharply inverted in leads II and III. In the cases in which precordial leads have been taken the curves obtained have been less distinctive than those described in the preceding paragraph. The first initial deflection has always been downward and it has usually been of large amplitude, particularly in those leads in which the exploring electrode was placed near the cardiac apex. The T-waves of the special leads were strongly inverted in cases in which T was sharply inverted in lead III; when there was an elevation of the S-T segment in lead III this occurred in the precordial leads also.

In the autopsied cases curves of the type described under (a) have been associated, with one possible exception, with infarcts located on the anterior or antero-lateral surface of the left ventricle. Curves of the type described under (b) have been associated with infarcts located on the posterior wall of the left ventricle. These findings are in agreement with those of Barnes and Whitten.[9] Many of the patients studied displayed curves which could be easily classified as belonging to one group or the other although they did not show all of the features described as typical of the group to which they obviously belonged. In other instances the electrocardiogram was definitely abnormal but showed no peculiarities that would suggest cardiac infarction.

Changes in heart rhythm, particularly the occurrence of partial or complete A-V block, paroxysmal tachycardia of ventricular origin, and the sudden development of intraventricular block, or of very small ventricular complexes are also common in coronary thrombosis and are of diagnostic value when the other data available are suggestive.

[9] Barnes, A. R., and Whitten, M. B., *Am. Heart J.*, 1929, **5**, 142.

6191

Observations on Heart Sounds with Particular Reference to Gallop Rhythm and Sounds of Auricular Origin.

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The study of a large number of phonocardiograms from a series of patients with heart disease and from normal subjects suggests the tentative conclusions enumerated below. Two Einthoven galvanometers coupled in tandem were used to record the electrocardiogram and the heart sounds simultaneously and the electric stethoscope manufactured by the Western Electric Company was used to convert the sounds into electrical variations.

From the graphic standpoint gallop rhythm may be defined as a condition in which 3 sounds occur during each cardiac cycle; 3 or perhaps 4 varieties may be distinguished.

(a) Protodiastolic gallop rhythm in which the extra sound follows the second heart sound by a constant interval and occupies the same position in the cardiac cycle as the normal third heart sound.

(b) Presystolic gallop rhythm in which the extra sound precedes the first heart sound by a constant interval, and is almost certainly of auricular origin. The onset of the extra sound falls within the P-R interval of the electrocardiogram. This appears to be the most common type of gallop rhythm; it is particularly frequent in cases of arterial hypertension.

(c) Systolic gallop rhythm in which the extra sound falls within the limits of ventricular systole. This type of gallop rhythm is uncommon and apparently has no clinical significance.

(d) Gallop rhythm due to audible auricular sounds when the P-R interval of the electrocardiogram is increased beyond normal limits in which case the extra sound may fall in any part of diastole. This should probably be considered a variety of presystolic gallop rhythm since the extra sound is linked to the first heart sound which follows auricular systole by a constant interval so long as there is no dropping out of ventricular beats.

In 3 cases of bundle branch block with gallop rhythm the extra sound occurred in presystole and preceded the first heart sound by a constant interval. The extra sound could not therefore be attributed to asynchronous contraction of the 2 ventricles. In a fourth case of

bundle branch block no gallop rhythm was present; the first and second heart sounds showed normal time relations.

We have seen one instance in which 2 extra sounds, one of which was linked to the first and the other to the second heart sound fell together at the usual heart rate and gave rise to a very pronounced gallop on auscultation. When the heart rate was slowed by pressure upon the carotid artery these 2 sounds were separated.

In complete and in high grade partial heart block auricular sounds are as a rule easily recorded. Very frequently each auricular contraction produces 2 sounds. The intensity of the auricular sounds varies greatly with the relation of auricular to ventricular systole; they are often very loud when the P-deflection of the electrocardiogram falls near the end of the T-wave. In one case of complete heart block auricular sounds occurred during ventricular systole as well as in diastole, indicating that auricular contraction may produce a sound even when it expels no blood into the ventricles.

In some cases of complete heart block the R-T interval of the electrocardiogram is nearly twice as long as the length of systole measured from the beginning of the first to the beginning of the second heart sound. Less pronounced discrepancies between the R-T interval and the length of mechanical systole were observed when the cardiac mechanism was normal.

6192

Electrocardiographic Leads Which Record Potential Variations Produced by the Heart Beat at a Single Point.

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In a recent publication[1] we have shown that if the assumptions upon which Einthoven's equilateral triangle is founded are valid the potential of the right arm (V_R), the potential of the left arm (V_L), and the potential of the left leg (V_F) at any given instant in the cardiac cycle are defined by the following equations:

[1] Wilson, F. N., Macleod, A. G., and Barker, P. S., *Am. Heart J.*, 1931, **7**, 207.

$$V_R = -\frac{e_1+e_2}{3} \qquad (1)$$

$$V_L = \frac{e_1-e_3}{3} \qquad (2)$$

$$V_F = \frac{e_2+e_3}{3} \qquad (3)$$

where e_1, e_2, e_3 represent the deflections in standard leads I, II and III respectively at the chosen instant. It will be seen that at every instant

$$V_R + V_L + V_F = 0. \qquad (4)$$

Let suitable electrodes be placed upon the right arm, left arm and left leg in the same manner as in taking ordinary electrocardiograms and let these electrodes be connected, each through a resistance R, to one and the same terminal. Providing that the 3 resistances (R) are equal and sufficiently great so that this procedure does not materially alter the potential variations produced by the heart beat at the extremities, it may be shown that the potential of this central terminal will not be significantly influenced by the heart beat and may be taken as zero throughout the cardiac cycle. If an instrument which has a resistance sufficiently high so that it may be regarded as a potentiometer is connected to this terminal and to any body point, it will record the potential variations produced by the heart beat at the latter point. In this way V_R, V_L and V_F may be directly recorded by connecting the recording instrument to the central terminal and the electrode in contact with the proper extremity, and the characteristics of the electric field set up in the body by the heart beat may be easily investigated. Lead V_L, as indicated by equation (2), gives a direct measure of the index employed by Lewis,[2] White[3] and others for determining the grade of right or left preponderance present.

The statement that the potential of the central terminal is not influenced by the heart beat is easily proved in the following way. Let V_0 represent the potential of this terminal. If the currents flowing from the right arm, the left arm and the left leg toward the central terminal are represented by I_R, I_L, and I_F respectively, we have the equations

$$V_0 = V_R - RI_R \qquad (5)$$

$$V_0 = V_L - RI_L \qquad (6)$$

$$V_0 = V_F - RI_F \qquad (7)$$

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[2] Lewis, Thomas, *Heart*, 1913-14, **5**, 398.

[3] White, P. D., "Heart Disease", The Macmillan Co., New York, 1931.

By adding these equations we obtain

$$3V_0 = V_R + V_L + V_F - E(I_R + I_L + I_F) \qquad (8)$$

The expression enclosed in parenthesis is obviously equal to zero by Kirchhoff's first law, which states that in any network the algebraic sum of the currents meeting at a point is zero. Consequently, by equation (4) the right hand member of equation (8) is equal to zero and V_0 is not influenced by the heart beat.

In our experiments we have connected each of the 3 extremities to the central terminal through a non-inductive resistance of 25,000 ohms. In order to convert the string galvanometer into a potentiometer we have inserted a vacuum tube in the string circuit. In doing this we have adopted the method employed in Dr. W. J. V. Osterhout's laboratory and we wish to thank Dr. S. E. Hill for his kindness in supplying us with the necessary data.

6193

Cultivation of Herpes Virus, and Use of the Mouse in its Titration.

R. S. SADDINGTON. (Introduced by Simon Flexner.)

From the Laboratories of The Rockefeller Institute for Medical Research, New York City.

Parker and Nye[1] attempted, unsuccessfully, to cultivate the herpes virus. Rivers[2] and his coworkers, using rabbit cornea embedded in rabbit plasma, were, however, more successful, and later, Gildemeister[3] and his associates grew the virus over 22 successive generations, their medium being rabbit testicle in rabbit plasma. The most recently published work in this connection has been that of Andrewes,[4] who reported successful cultivation of the herpes virus over 23 successive generations.

We have recently successfully cultivated the H. F. strain of herpes virus through 25 generations. The medium used has been similar to that of Andrewes. Three cc. of Tyrode's solution and

[1] Parker, F., Jr., and Nye, R. N., *Am. J. Path.*, 1925, **1**, 337.

[2] Rivers, T. M., Haagen, E., and Muckenfuss, R. S., *J. Exp. Med.*, 1929, **50**, 665.

[3] Gildemeister, E., Haagen, E., and Scheele, L., *Zentr. f. Bakt.*, Abt. 1, 1929, **114**, 309.

[4] Andrewes, C. H., *J. Path. and Bact.*, 1930, **33**, 301.

1 cc. of normal rabbit serum were placed in small River's flasks, to which were added fragments of finely minced fresh rabbit testicle. The original inoculum was 0.2 cc. of a 10% emulsion of brain from a rabbit which had succumbed to typical herpetic encephalitis. In making subsequent transfers from one generation to another, 0.2 cc. of the preceding culture was inoculated into freshly prepared flasks. These flasks were incubated for 4 days.

In testing for the presence of virus, corneal inoculations were used. Inasmuch as it is desirable to have confirmation of growth other than extended numbers of passages, it was deemed pertinent to attempt the use of mice as a means of titration. The susceptibility of mice has, of course, been known for some time. Blanc and Caminopetros,[5] Doerr and Schnabel,[6] and Flexner and Amoss[7] first showed that a fatal encephalitis in mice followed appropriate intracranial inoculations. The most recently reported work with mice has been that of Andervont.[8, 9]

Preliminary experiments with our cultures revealed that the cultivated virus produced an encephalitis in mice in dilutions up to 1:500. Inasmuch as in the higher dilutions symptoms were delayed and the period of illness prolonged, an arbitrary choice of dilutions for titration was made. Thus, each generation of the cultures being tested was inoculated intracranially into mice, undiluted and in dilutions of 1:10 and 1:100. The dose was never more than 0.05 cc. For each 3 mice inoculated a fourth was injected with saline only, to serve as a control.

Two different cultures of the H. F. strain were thus titrated over a considerable number of generations. For the dilutions 1:10 the average number of days of survival following the inoculation was for one culture 3.9 days and for the other 3.7 days. In the dilutions of 1:100 the survival periods were 4.6 days and 4.5 days respectively. These figures fall within the limits recorded by Andervont, who reported an average incubation period of 3 days.

As well as cultivation *in vitro*, growth of the herpes virus *in vivo* has been accomplished, the chick embryo serving as host. The technique of inoculating the chorio-allantoic of the chick was first described by Clark,[10] and has more recently been utilized by Good-

[5] Blanc, G., and Caminopetros, J., *Compt. rend. Soc. biol.*, 1921, **84**, 859.

[6] Doerr, R., and Schnabel, A., *Ztschr. f. Hyg. u. Infektionskr.*, 1921, **94**, 29.

[7] Flexner, S., and Amoss, H. L., *J. Exp. Med.*, 1925, **41**, 233.

[8] Andervont, H. B., *J. Inf. Dis.*, 1929, **44**, 383.

[9] Andervont, H. B., *J. Inf. Dis.*, 1931, **49**, 507.

[10] Clark, E. R., *Science*, 1920, **51**, 371.

pasture[11, 12] and his associates, and by Rivers[13] and his coworkers. Goodpasture was the first to observe that the virus of herpes simplex was capable of infecting chick membranes. We have succeeded in cultivating the herpes virus in this way through 24 generations. Ten-day chicks were used, and transfers were usually made every 4 days, small pieces of infected chick membrane serving for inoculation of succeeding generations.

The presence of and the growth of the virus was demonstrated by means of corneal inoculation in the rabbit, by histological examination of the chick membranes and by means of mice titration. For this last purpose, infected chorio-allantoic membrane was finely minced in fluid taken from the infected embryos. The liquid material thus obtained was inoculated intracerebrally into mice, undiluted and in dilutions of 1:10 and 1:100. Sterile saline was used as a diluent. The amount inoculated was never more than 0.05 cc. For 11 generations thus titrated the average survival periods were 5.5 days, and 6.5 days for the 1:10 and the 1:100 dilutions respectively, results which conform with those recorded above.

As a result of this work it may be concluded that the herpes virus, the H. F. strain of which was used, can be successfully cultivated *in vitro* and *in vivo* and that the mouse can be successfully employed as an indicator of maintained activity of the virus thus grown.

6194

Ineffectiveness of Certain Pentavalent Arsenicals Used Orally in T. hippicum Infected Guinea Pigs.

H. H. ANDERSON.

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Kolmer[1] has reported that "stovarsol and treparsol in doses of approximately 0.030 to 0.040 gm. per kilo of weight by oral administration for 3 to 10 days were effective in preventing trypanosomiasis of rats infected with *T. equiperdium.* Atoxyl was slightly

[11] Woodruff, A. M., and Goodpasture, E. W., *Am. J. Path.*, 1931, **7**, 209.

[12] Goodpasture, E. W., Woodruff, A. M., and Buddingh, G. J., *Science*, 1931, **74**, 371.

[13] Rivers, T. M., and Schwentker, F. F., *J. Exp. Med.*, 1932, **55**, No. 6, in press.

[1] Kolmer, J. A., with A. M. Bole, *Am. J. Trop. Med.*, 1931, **2**, 261; and *J. Pharm. and Exp. Therap.*, 1931, **43**, 521.

more effective, as doses of 0.020 to 0.030 gm. per kilo per day (orally) . . . . for 5 to 10 days prevented infection." Tryparsamide and "Bayer 205" or "Germanin" were ineffective when given orally to infected animals. He further states that when treatment was started 24 hours after infection the arsenicals were more active than when given before or at the time the infection was induced. Cooper[2] originally demonstrated this phenomenon using tryparsamide, etharsanol and "arsenoxide".

The treatment of equine trypanosomiasis *(T. hippicum)* has been unsatisfactory with the common trypanocides because horses require large amounts of expensive drugs given intravenously. Dr. Herbert Clark[3] of Panama has been partially successful with approximately 5 times the human dose of tryparsamide or "Bayer 205" combined with tartar emetic, given at weekly intervals over relatively long periods. Certain disadvantages are: first, that the cost of medication may exceed the value of the treated animal, secondly, that embolic phenomena occur with intravenous administration, thirdly, that neither prophylactic therapy nor treatment of infected animals is always successful. A more satisfactory form of therapy is needed, that is, a cheap drug that can be given by mouth without toxicity in prophylactic or therapeutic doses. Kolmer's report suggested the possibility of a successful approach to the problem from the standpoint of oral effectiveness with certain arsenical trypanocides.

Guinea pigs infected with the Panamanian strain of *T. hippicum* were treated with atoxyl, acetarsone ("stovarsol"), and carbarsone given orally. Standard intraperitoneal injections were made into normal healthy guinea pigs using 0.3 cc. of infected guinea pig blood with an equivalent amount of sterile physiological saline. The infection in the control group of untreated pigs caused death usually in one month, but a few animals survived without treatment for 6 weeks. No spontaneous cures were seen. Atoxyl was given orally in a total dose of 60 to 200 mg. per kilo in 5 divided amounts over 10 days. Acetarsone was given in the same manner in 200 to 300 mg. per kilo doses, while carbarsone was tried in 150, 250, and 300 mg. per kilo total dosage in 10 days' time. Ten animals were treated at each dose and a number of similarly infected but untreated controls were used for comparison in each group. Treatment was started 24, 48, and 72 hours after the trypanosomes had been injected intraperitoneally. The only favorable response noted was a gain in weight in the treated guinea pigs. There was no perma-

[2] Cooper, G. A., *J. Pharm. and Exp. Therap.*, 1930, **29**, 255.

[3] Clark, Herbert C., personal communication.

nent clearing of the blood stream of trypanosomes during the follow up period, and death occurred on the average in the treated group as quickly as in the untreated controls. No treated animal survived longer than 6 weeks after it became infected.

Summary. Atoxyl (in 60 to 200 mg. per kilo), acetarsone (in 200 to 300 mg. per kilo), and carbarsone (in 150, 250, and 300 mg. per kilo) were given orally without significant effect to guinea pigs experimentally infected with *T. hippicum.*

6195

Comparative Protective Efficiency of Some Barbitals Against the Symptoms of Anaphylactic Shock.

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It is well known that certain depressants of the central nervous system are effective in preventing fatal anaphylactic shock. Many of them, however, would not be as good protective agents against the symptoms of shock in sensitized individuals as would certain hypnotics. We have, therefore tried out the efficiency of some of the barbitals for this purpose. Three representative members of this group were selected for study: amytal, phenobarbital, and barbital. The experimental evidence here recorded supports the conclusion that phenobarbital alone possesses a highly protective efficiency against the symptoms of anaphylactic shock.

Experiments were carried out on 61 animals divided as follows: 14 controls, 10 injected with amytal, 14 with barbital, and 23 with phenobarbital. Guinea pigs weighing 250 to 300 gm. were sensitized by the intraperitoneal injection of 1 cc. horse serum. After an incubation period of 2 to 3 weeks, an intracardiac injection of a second dose of serum usually produced typical anaphylactic shock with rigid distention of the lungs in about 86% of the controls. These control observations as well as those recorded in previous communications,[1, 2] have satisfied us that the intraperitoneal route

[1] Hurwitz, S. H., and Nicholls, E. G., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 139.

[2] Hurwitz, S. H., and Wessels, A. L., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 120.

for sensitization and the intracardiac route for shock injections give a high percentage of positive results.

The sodium salts of the barbitals were administered intramuscularly: amytal from 50 to 70 mg., barbital 80 to 200 mg., and phenobarbital 40 mg., per kilo of body weight. These doses were found to be nonfatal and definitely depressant for guinea pigs, though very large compared to single therapeutic doses in man. The effects produced corresponded to the ordinary hypnotic action of the drugs with more marked depression and some anesthetic effect in animals given daily injections of amytal and the larger doses of barbital. In only a few instances was the drug effect sufficient to mask the symptoms of anaphylactic shock. About 20 minutes was allowed for the preliminary injection of the barbitals before giving the intracardiac injection of the second dose of serum.

Results. 90% of the guinea pigs given injections of amytal showed the typical signs of anaphylactic shock and died in about 5 minutes. Those animals given daily cumulative doses of amytal for a week showed no more resistance to shock than those which received the single doses. Of the 14 animals receiving barbital, 77% died in typical anaphylactic shock; whereas only 5 of 23 animals given phenobarbital, or 22%, succumbed. That the survivors passed through mild shock and were therefore sensitized was shown by the presence of some of the typical symptoms: restlessness, convulsions, dyspnea and rubbing of the nose. Compared with a control mortality of 86%, a reduction of the mortality to 22%, or approximately one-fourth, by the administration of phenobarbital is impressive.

In man, our experience with phenobarbital in anaphylactic conditions has been limited to a small number of patients with allergic and intractable asthmas. In them, the oral administration of phenobarbital in therapeutic doses has been found to prolong the relief from the symptoms of bronchospasm obtained by epinephrine or ephedrine. A combination of ephedrine and phenobarbital has been found particularly useful in the intractable asthmatic who reacts unfavorably to ephedrine products.

6196

An Improved Method of Protecting the Peritoneum of Dogs against Fatal Colon Bacillus Infection.*

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The method here described produces a protection of the peritoneum against a fatal infection by means of a single intraperitoneal injection. The maximum protection is obtained in 48 hours with some degree within 12 hours. The protection produced is against any of the organisms that are common to the gastro-intestinal tract. Goldblatt and I[1] observed that *B. coli* suspended in physiological sodium chloride and injected intraperitoneally passed rapidly from the peritoneal cavity into blood and lymph. However, *B. coli* suspended in gum tragacanth was found to be retained to a marked degree within the peritoneal cavity. Subsequent experiments[2, 3, 4, 5, 6] demonstrated that peritoneal protection against a fatal infection can be achieved by the production of a sufficiently large number of polymorphonuclear phagocytes which appear rapidly. This process was designated as hyperleukocytic pre-immunity and the protection was assumed to be due to a coincident presence of phagocytes evoked by the introduction of the material.

In previous experiments peritoneal protection was achieved by 4 intraperitoneal injections of *B. coli* suspended in physiological sodium chloride on successive days with a survival of 65% of the animals. Since the peritoneal protection was found to be due to a local migration of polymorphonuclears and, since *B. coli* in physiological sodium chloride passed from the peritoneal cavity but were retained when suspended in gum tragacanth, it was deduced that a suspension of *B. coli* in gum tragacanth may constitute a more efficient protecting substance.

Thirty-two dogs were given single intraperitoneal injections of one billion heat killed *B. coli* (culture 300) suspended in 1% gum

* This work is aided by a grant by the Committee on Scientific Research of the American Medical Association.

1 Steinberg, B., and Goldblatt, H., *Arch. Int. Med.*, 1927, **39**, 449.

2 Steinberg, B., and Snyder, D. A., *Arch. Path.*, 1929, **8**, 419.

3 Steinberg, B., *Arch. Surg.*, 1931, **23**, 145.

4 Steinberg, B., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 16.

5 Steinberg, B., PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 18.

6 Steinberg, B., *Arch. Surg.*, 1932, **24**, 308.

tragacanth (bacteria were killed at 80°C. for 10 minutes). At intervals of 12, 24, 48, and 72 hours following the protecting injections, a peritonitis was induced by the intraperitoneal injection of living *B. coli* suspended in 2½% gum tragacanth. Twenty-five control dogs were given similar injections of living *B. coli* in gum tragacanth. All the control dogs died. The percent of survivors of protected dogs was 40, 60, 80 and 75% respectively.

In spite of the peritonitis which was overwhelming in character and sudden in onset, the best survival percentage is 80 as contrasted with 65% by the former method. The material used for the production of peritonitis is several times the lethal dose for a dog of average weight.

Other experiments indicate that there is a quantitative relationship between the amount of the protecting substance and the percentage of surviving animals. There was a decreasing number of surviving dogs with a decrease in the number of bacteria composing the substance. These experiments also demonstrate that the gum tragacanth itself is not an important factor in the production of the peritoneal protection.

It was demonstrated previously[7] that the use of *B. coli* (culture 300) as a protecting substance resulted in survivals of animals with peritonitis induced by introduction of feces containing many and varied bacteria. Experiments with the protecting substance here described confirm the formerly expressed view.

Examination of the peritoneum of the animals injected with the protecting substance did not reveal presence of adhesions.

6197

Homolateral Synchronism of Lymphatic Hearts.

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It is a long recognized fact that the opposite members of each of the 2 pairs* of anuran lymph hearts do not beat in unison. There has apparently resulted a universal assumption that the same lack

[7] Steinberg, B., and Goldblatt, H., *Arch. Int. Med.*, 1928, **42**, 415.

* The posterior pair is of multiple origin, and in certain of the Salientia retains the separate character.

of unison exists between the anterior and posterior members of these pairs; that the several automatic spinal centers, which are known to deliver groups of nerve impulses rhythmically to the lymph hearts, operate each independently of the others[1] (Fig. 1, *a*).

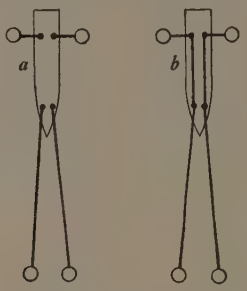

FIG. 1.

a. Schema illustrating the general concept of independent spinal automatic centers actuating the lymph hearts. *b*. Completion of the schema, expressing the discovered integration of homolateral centers. Black circles indicate spinal centers; circles in outline, the anterior (subscapular) and posterior (coccygeal) pairs of hearts.

Such independence, however, is discovered to be purely contralateral. A simultaneous mechanical record of the beats of anterior and posterior hearts *of the same side* reveals a strict correspondence of rhythm, in contrast to the obvious lack of synchrony registered at the same time by a heart of the opposite side (Fig. 2). The synchrony is remarkably stable, persisting even in extreme arrhythmia (Fig. 3). Spinal section between 4th and 5th vertebrae

FIG. 2.

Simultaneous heart-lever tracings from lymph hearts of bullfrog *(R. catesbiana)*: *a*, right anterior; *b*, right posterior; *c*, left posterior. Time in seconds.

FIG. 3.

Synchronous beats with arrhythmia induced by a rising antero-posterior temperature gradient *(R. pipiens)*: *a*, left anterior; *b*, left posterior. Time in seconds.

[1] For account of amphibian lymph hearts, with recent literature, see G. K. Noble, The Biology of the Amphibia, N. Y., 1931, 195.

abolishes the unison but not the activity of the homolateral organs, while curarization or stimulation of one fails to influence the other. The synchronizing influence is therefore of central and not of peripheral (proprioceptive) origin.

Evidence indicates that the regions of motor outflow to the lymph hearts of the same side, in both larval and adult forms, are connected (Fig. 1, *b*) through an intraspinal pacemaker system effecting an exclusively homolateral coordination.

6198

Excretion of Lactic Acid in Sweat.

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The reaction, osmotic pressure and volume relationship of the body fluids are closely dependent on the properly regulated acid-base equilibrium. Under normal conditions 800 to 1000 cc. of fluid are lost through the skin in 24 hours; while under special conditions such as athletic contests, heavy work such as mining, etc., this may be increased to as much as over 3 liters per hour. Even under normal conditions there is a much greater amount of acid claiming excretion than fixed base taken in. During exertion this lack of balance between fixed base and acid catabolites is augmented and some regulatory mechanism is essential if the fixed base depots of the body are not to be abnormally drained. We have attempted to investigate how some of these regulatory devices operate to conserve base within the body during the excess sweating which takes place in patients whose temperature is raised to high levels for therapeutic reasons. These patients are taken at random and subjected to this therapy for causes as varying as acute gonorrheal infections, paralysis, psoriasis, etc., so that the results obtained are probably generally applicable and not due to the specific pathological condition.

The base economy factor of the skin will depend on its ability to produce a fluid of lower pH than the blood plasma which acts as the carrier of the catabolic products. The sweat as collected from the surface of the entire body at intervals after the temperature of the patients has been raised showed a pH of about 4-4.5. It is a known fact that athletes complain of "stinging sweat". The pro-

duction of an acid that could be eliminated un-ionized to a low degree at the pH of sweat would result in a great sparing of base. We have found comparatively large quantities of lactic acid in the sweat of these patients. From a study of the dissociation constant of lactic acid it is apparent that at the pH of sweat lactic acid is approximately 50% ionized and hence can be secreted undissociated with the consequent sparing of base, especially of potassium. At the pH of sweat the buffer action of the lactates in the presence of an equivalent amount of lactic acid is at a maximum, thus preventing the fall of the pH of the sweat to harmful levels. A patient loses 2.5-5.0 liters of fluid during a treatment, excreting 250-300 mg. of lactic acid per 100 cc., which means a total excretion of as much as 15 gm. of lactic acid. Snapper and Gruenbaum[1] found that athletes excrete large quantities of lactic acid during races. Lactic acid was determined by the method of Clausen.[2]

A patient whose temperature was raised to 106° was found to have lost approximately 4 liters of fluid, exclusive of 122 cc. of urine. Certain of the chemical constituents of the blood and sweat are given in the following table.

TABLE I.

Constituent	Blood Before	Blood After 3 hr.	Sweat After 1 hr.	Sweat After 3 hr.
pH	7.45		4.15	4.12
CO_2	61.2	57.2	12.4	13.4
Lactic acid	21	72	216	254
Na	353	316	140.8	178
K	19	21	36.9	35.5

(All quantities with the exception of pH are mg. per 100 cc.)

6199

Some Differential Reactions in the Colon-Aerogenes Group of Bacteria.

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Two distinct subgroups are generally recognized in the colon-aerogenes group of bacteria: the genus *Escherichia* which grows poorly if at all on citric acid, is acid to methyl red (M.R.+), does

[1] Snapper, I., and Gruenbaum, A., *Bio. Zeitschr.*, 1929, **206**, 319.
[2] Clausen, S. W., *J. Biol. Chem.*, 1922, **52**, 263.

not produce acetyl-methyl-carbinol from glucose, (V.P.—), and is generally of fecal origin, and the genus *Aerobacter* which grows luxuriantly on citric acid, is alkaline to methyl red (M.R.—), produces acetyl-methyl-carbinol, (V.P.+) and is generally found in soil or on grains. Recently a number of strains have been reported which grow luxuriantly on citric acid but are negative as respects the V.P. reaction and frequently neutral or acid to methyl-red. The systematic position of these strains is in doubt.

Werkman and Gillen[1] have recently suggested the generic name, *Citrobacter* for bacteria producing trimethylene-glycol from glycerol, which can utilize citric acid but are V.P. negative and M.R. neutral or acid. Whether all of the strains falling in this category on the citric acid, V.P. and M.R. tests are capable of producing trimethylene-glycol is not known. The lack of a suitable, convenient, rapid and reliable test for trimethylene-glycol together with the questionable M.R. and V.P. reactions of these strains, renders the discovery of a simple differential test which will distinguish these cultures from typical *Escherichia* and *Aerobacter* highly desirable. The production of H_2S from Difco proteose peptone seems to serve this purpose admirably.

In the accompanying table are shown the results of a study of 401 strains of members of the colon-aerogenes group isolated from eggs. The percentages are considered tentative, as several additional cultures are under observation, and it is possible that some impure cultures might be detected before completion of the study. Only those characteristics which show distinct and clear-cut differential value are considered here.

TABLE I.

Some Differential Reactions in Colon-Aerogenes Group.

Genus	Escherichia	Aerobacter		
		cloacae	aerogenes	?
No. of Strains	155	80	123	43
Character	% Positive Reactions			
Citric Acid	0	100	100	100
V.P.+ M.R.—	0	100	100	0*
H_2S	1	0	0	100
Indol	97	0	60	0
Glycerol	47	0	100	100
Aesculin	73	34	100	0
Salicin	71	74	100	0
Starch	0	1	99	0
Pectin	1	6	90	0

* Frequently questionable in M.R. or V.P. reactions, but generally would be recorded. V.P.— M.R.+ or (?).

[1] Werkman, C. H., and Gillen, G. F., *J. Bact.*, 1932, **23**, 167.

It will be noted that 155 failed to utilize citric acid and are therefore considered as of the genus *Escherichia*. The remaining 246 strains are tentatively considered as *Aerobacter* because they did utilize citric acid. Of these, 80 failed to attack glycerol and are considered *Aerobacter cloacae*. Of the remaining 166 cultures, 123 were typical for the *Aerobacter aerogenes* group as respects the citric acid, V.P., M.R. and glycerol reactions. The 43 remaining cultures differed from the latter in that the V.P. and M.R. reactions were non-correlating with growth on citric acid. These were practically the only strains which produced H_2S, a characteristic which clearly differentiated them from all of the other groups under consideration. Furthermore, this group of 43 strains seems to be clearly differentiated from each of the other groups by a number of characters, as may be readily seen from the accompanying table. Generic or specific allocation is withheld pending determination of ability to produce trimethylene-glycol from glycerol.

Determinations of H_2S were made with a number of different media and indicators, including iron (ferric chloride), nickel, lead, and manganese salts. Difco proteose peptone (2%) 0.1% K_2HPO_4 in agar with iron citrate (0.05%) as the indicator was found to be very sensitive, definite tests for H_2S, as indicated by blacking along the line of inoculation, being observed in as short a time as 12 hours, and very strong tests in 24 to 48 hours at 37°C.

6200

Endocrine Reactions of X-Ray Sterilized Males.*

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From the State University of Iowa.

Investigations in the field of testicular physiology have borne out that the germinative and the endocrine systems are highly independent of each other, despite the obvious parallelism in their embryonic development. Cryptorchidism and X-ray sterilization result in many cases in complete degeneration of the germ cells, without affecting, however, the endocrine system, as evidenced by the normal development and persistence of the secondary sex characters

* This investigation was supported by grants from the Committee for Research in Problems of Sex and by the Committee on Effects of Radiation, National Research Council.

(seminal vesicle and prostate tests). On the other hand, Moore and Samuels[1] recently have shown that vitamin B deficiency inhibits selectively the endocrine system so that the male rats assume the castrate type while spermatogenesis proceeds normally.

The close time correlation in endocrine and spermatogenetic development is simply due to the fact that both are under the control of the hypophysis. The hypophysis stimulates the testicular development and answers with an increased hormone production if the testes are ablated. This reaction is most strikingly demonstrated in parabiosis experiments. Hill[2] has shown that the normalcy of the oestrus cycles of a rat female is undisturbed by a male parabiont. After castration of the male, however, the female falls into anoestrus for a period and then goes into permanent oestrus. These reactions are apparently due to the influx of excessive amounts of hypophysis hormone from the castrate, which initially cause luteinization of the ripe follicles and later the formation of cystic follicles.

One has generally been inclined to assume that the hyperactivity of the hypophysis is an answer to the deficiency or the complete absence of the endocrine component of the testis. It appeared of interest, therefore, to determine whether the hypophysis responds also to the absence of the germinal component only.

A series of 9 rat males, 2 months old, were sterilized, by the administration of 2400 r units of X-rays in 3 treatments. One month later they were united in parabiosis with normal females. Following this operation all the females exhibit irregularities in their oestral cycles; the oestrus periods are prolonged and eventually become permanent. The reaction differs only quantitatively from that observed in females united with totally castrated males. Hence it follows that X-ray sterilization causes a hyperactivity of the hypophysis. Pair 119 was selected for a thorough investigation. A colorimetrical test proved that the blood exchange between the X-rayed male and the female was of a high average value. The testicles measured 11 x 6 x 6 mm., their weight and volume being about 25% that of normal controls. The epididymes and Cowper's glands were of normal size, the prostate and seminal vesicles distinctly enlarged. Cross sections through the testes give the usual picture of X-rayed testes with the relatively abundant interstitium and absence of germ cells.

There remains to consider whether the decrease in size of the

[1] Moore, C. R., and Samuels, L. T., *Am. J. Physiol.*, 1931, **96**, 278.

[2] Hill, *J. Exp. Zool.*, 1932, in press.

testis might include a reduction of its endocrine component, which could possibly be the cause of the increased hypophyseal activity. Facts are not in favor of this interpretation. The interstitial cells appear normal in quality and quantity and the fact that the seminal vesicles and the prostates of our irradiated male are rather above the normal size clearly indicates that the endocrine system remained unimpaired by the amount of X-rays administered. We conclude, therefore, that the lack of spermatogenetic activity alone is responsible for the increase in hypophyseal activity in the X-ray sterilized male rat.

6201

Biological Assay of Pregnandiol.

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From the Laboratories of Mount Sinai Hospital, New York City.

Through the kindness of Dr. A. Butenandt of Göttingen who supplied us with 500 mg. of crystalline Pregnandiol[1] obtained from pregnancy urine, we were able to subject the substance to physiological tests. Pregnandiol, of which from 0.1 to 0.2 gm. can be obtained from 100 liters of human pregnancy urine, is, according to Butenandt, a saturated di-secondary alcohol of the formula $C_{21}H_{24}(OH)_2$ containing 4 hydrated rings in the molecule.

The tests performed were as follows:

a. Injected (in oil) into castrated mice up to the dosage of 10 mg. it shows no female sex hormone effects (as already determined by Butenandt).[1]

b. Injected into immature rats up to the dose of 30 mg. it produces no ovarian changes characteristic of the prepituitary hormone.

c. Injected for 5 days into castrated rabbits primed for 5 days with female sex hormone, it produces no progestin (corpus luteum) effect on the uterus with a total dosage of 150 mg.

d. Injected into the virgin immature castrated guinea pig primed with female sex hormone up to a dosage of 100 mg., it fails to cause relaxation.

e. Injected into the castrate mouse brought to estrus by means of female sex hormone, it shows no mucifying effect on the vaginal epithelium.

[1] Butenandt, A., *Berichte d. deut. chem. Gesellschaft*, 1930, **63**, 659; 1931, **64**, 2529. Marrian, G. F., *Biochem. J.*, 1929, **23**, 1090.

It therefore appears that Pregnandiol is physiologically inert as far as the female sexual tract is concerned.

6202

Influence of Sex Hormones on the Occurrence of Tissue Macrophages in the Rabbit's Uterus.*

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It has been shown[1] that during pregnancy there is a great increase in the number of mononuclear phagocytic cells in the wall of the rabbit's uterus. This report is of an attempt to determine the extent to which this phenomenon might be attributed to hormonal factors, and is based on results obtained with over 70 female rabbits. In all instances the macrophages were demonstrated by intravital staining. Each animal was given intravenously from 20 to 25 cc. per kilo body weight, of a 1% aqueous solution of Trypan Blue, over a period of 3 days. They were sacrificed on the fourth day, and paraffin sections of the uteri were made using alum carmine as the counterstain.

It was found that (1) in the uterus of the normal unmated rabbit small numbers of faintly-staining macrophages are generally present, while they are completely absent in the atrophic uterus of spayed animals, and (2) the induction of progestational proliferation in the rabbit's uterus by the intravenous injection of urine from pregnant women or of estrin-free ovary-stimulating extracts made from blood of pregnant patients, does not generally cause an increase in the number of macrophages. This, however, was demonstrated in a few instances.

The effect of various hormonal conditions on the response of the uterus to trauma was then determined. The injury was produced by the method of Long and Evans[2] for the experimental production of placentomata, namely by the introduction of a silk ligature

* Supported by a grant from the Rockefeller Fluid Research Fund of the Stanford University School of Medicine.

[1] Fluhmann, C. F., *Am. J. Obstet. and Gynec.*, 1928, **15**, 783.

[2] Long, J. A., and Evans, H. M., The Oestrous Cycle in the Rat, Mem. Univ. California, 1922.

through one horn of the uterus. The results showed that: (1) In normal rabbits and in spayed animals large numbers of macrophages soon appeared at the site of the injury, but this response was essentially of a local nature and there was no great increase of the cells at a distance from the ligature or in the opposite uterine horn, but (2) in the animals in which progestational proliferation had been induced, the reaction to an irritant, such as a silk ligature placed in one horn, was greatly increased. In this case large numbers of deeply-staining macrophages were found not only at the site of the injury but throughout both uterine cornua.

It is thus apparent that when the rabbit's uterus undergoes the tissue differentiation and vascular changes induced by the ovarian hormones estrin and progestin, it acquires the power of responding much more actively with macrophages to traumatic stimuli.

During pregnancy in the human, fixed macrophages are found in large numbers in the uterus (Hornung[3]), and in the broad ligament (Hofbauer[4]), and they are generally considered as important factors in the defensive mechanism against local infection. It would seem justifiable, therefore, to attempt the treatment of pelvic inflammatory conditions with ovary-stimulating hormones, as originally recommended by Klein.[5]

6203

Further Studies on Immunity to Diphtheria Among Central and Polar Eskimos.*

J. RALPH WELLS AND PETER HEINBECKER.

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Inasmuch as immunity to various diseases is often found among persons in whom no previous exposure to infection has been established, several authors have postulated the existence of a physiolog-

[3] Hornung, R., *Zentrlbl. f. Gynaek.*, 1924, **48**, 2170.

[4] Hofbauer, J., *Bull. Johns Hopkins Hosp.*, 1926, **38**, 255.

[5] Klein, P., *Z. f. Geburtsh. u. Gynaek.*, 1929, **95**, 371.

* This work was made possible by a grant made by the Rockefeller Foundation to Washington University for research in Science, and through the cooperation of the Department of the Interior, Northwest Territories and Yukon Division, Ottawa, Canada.

ical process of "serological ripening" to explain this phenomenon.[1, 2, 3, 4, 5]

The widespread immunity to diphtheria among Eskimos was repeatedly quoted as an instance of such phenomenon and seemed particularly suitable for study. Materials collected by one of us (P.H.) from 7 groups of these people in 1930 were examined, and the results already reported[6] indicated that it was a true antitoxic immunity, and suggested the importance of carriers. Additional materials were collected in 8 such communities during the summer of 1931 by one of us (P.H.) and examined.

From the 214 available throat cultures, 34 strains of diphtheria-like organisms were isolated, but it was not possible to demonstrate toxin production at the time of isolation (after 60-80 days maintenance on artificial media). Three strains were morphologically and culturally typical (apparently "R") organisms, while the remainder gave atypical fermentation reactions. The throat flora in general was similar to that found in persons living in other latitudes.[7, 8]

The results of Schick tests (Table I) and serum antitoxin titrations (Table II) showed in general a somewhat higher incidence of susceptibility (Schick positive) than is found among persons living in regions where clinical diphtheria is more common. This is seen by a comparison of these results with the included Schick figures, compiled from von Groer's data[9] for Vienna and the antitoxin titrations of a St. Louis group, respectively.

TABLE I.

Group	Year	No. tested	% Schick Positive 3-18 yrs.	% Schick Positive 18 yrs.	% Schick negative 3-8 yrs.	% Schick negative 18 yrs.
Eskimos	1931	110	38	39	62	61
% of whole group				38		62
Vienna	1919		32	37	68	63
% of whole group				33		67

TABLE II.

Group	Year	No. tested	No. possessing antitoxin and units per cc. None	1/90	1/90	1/66	1/45	1/22	1/9	1/4	1/4
Eskimos	1931	39	18		1	1	1	3	8	5	2
St. Louis	1932	48	20	4	7	3	2	2	2	4	4

[1] von Groer, *Verhand. der Gesellsch. f. Kinderheilk.*, 1913, **30**, 182.
[2] Hirszfeld *et al.*, *Klin. Woch.*, 1929, **55**, 1046.
[3] Heinbecker and Irvine-Jones, *J. Immunol.*, 1928, **15**, 395.
[4] Bay-Schmith, *Klin. Woch.*, 1929, **8**, 974.
[5] Friedeberger *et al.*, *Deut. med. Woch.*, 1929, **55**, 132.
[6] Wells and Heinbecker, PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 887.
[7] Shibley *et al.*, *J. Exp. Med.*, 1926, **43**, 415.
[8] Bloomfield, *Johns Hopkins Hosp. Bull.*, 1921, **32**, 33.
[9] von Groer *et al.*, *Z. f. Immunität.*, 1919, **28**, 327.

In the few instances (18) where the amount of serum available was sufficient, adequately controlled complement-fixation tests were performed by the method of Krah and Witebsky,[10] using a lecithinized alcoholic extract of a mixture of 8 known virulent strains of *C. diphtheriae*. Five of these sera gave complete fixation. After absorption with known diphtheria organisms they showed no fixation, suggesting the presence of a specific antibody for *C. diphtheriae*.

Since it has been suggested that immunity to diphtheria is genetically associated with blood groups[2] we secured Schick data and blood groupings in 17 families (83 persons), but our evidence does not support the view of such a linkage.

Our findings indicate that resistance to diphtheria among Eskimos in these localities depends on the presence of antitoxin and suggest that carrier conditions, as well as latent specific infections, are the probable sources of this immunity.

6204

Nucleinate-Induced Extramedullary Myelopoiesis.

CHARLES A. DOAN.

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Experimental studies[1] established the direct action of the nucleinate molecule and of the nucleotides comprising it on the myeloid centers in bone marrow. Peripheral leucocytoses sometimes reaching 100,000 per cu. mm. and persisting for 3 to 4 days followed large single intravenous dosages (1 gm.) of these materials in normal rabbits.

More recent studies[2] have shown that daily injections of 0.35 gm. each of the pentose nucleotides in rabbits for a brief period (5 to 7 days) will produce an hyperplasia of the myeloid tissues in marrow, as proved by serial biopsies, with a definite absolute increase in granulocytes in the general circulation. During the succeeding 7 weeks the peripheral count returned gradually to the preinjection level and the bone marrow resumed its normal myeloplasia.

[10] Krah and Witebsky, *Z. f. Immunität.*, 1930, **66**, 59.

[1] Doan, C. A., Zerfas, L. G., Warren, S., and Ames, O., *J. Exp. Med.*, 1928, **47**, 403.

[2] Doan, C. A., *J. Am. Med. Assn.*, 1932, in press.

The nucleinate molecule, thus apparently possessing the property of direct myelopoietic stimulation, has been utilized to determine the inherent capacity of normal rabbit's marrow to respond under circumstances of long continued maximal stimulation. It was desired to test especially the stability of the normal mechanism of maturation and delivery and to see if an uncontrolled marrow hyperplasia with a leukemic arrhythmia in the peripheral blood could be made to supplant the more orderly processes observed in the acute leucocytic responses.[3] Or, it was thought that under such a continuous bombardment it might be possible to exhaust the ability of the marrow to respond with the gradual development of a relative hypoplasia or aplasia of the myeloid elements.

After observing 2 normal female rabbits (0-80 and 0-81) for one month, while adequate base line determinations of temperature, weight and blood cytology assured their suitability for this study, the daily intravenous injection of sodium nucleinate was started. The solutions were made up each day in normal saline prepared from fresh glass distilled water. Beginning with a dosage of 50 mg., maintained for one week, the amount was increased to 100 mg. Thereafter the daily dosage was increased 100 mg. each 7 days until 500 mg. was reached. After a 2 weeks' period at this level, the dose was raised by 100 mg. each week until the daily dose was 1 gm. Rabbit 0-81 received in all 56 gm. of sodium nucleinate over a period of 102 days and Rabbit 0-80 received 68 gm. over a period of 116 days before death ended the observations (May 1931). Both animals remained in apparent health throughout the experiment, gaining consistently in weight, in one instance from 1600 to 2300 gm. (0-80) and in the other from 1490 to 2030 gm. (0-81). The rectal temperatures at all times were within the limits of normal for the rabbit (100-102°). From a preliminary level of 3200 to 3900 neutrophilic granulocytes with a total count ranging between 8000 and 10,000 per cu. mm., the highest number of leucocytes recorded in Rabbit 0-80 was 58,300 with 49,500 neutrophils. The preliminary zonal range of neutrophils in Rabbit 0-81 was 960 to 2010 in a total leucocyte count of 6700 to 7400. The highest recorded count of white blood cells in this animal was 45,950 with 36,300 neutrophilic granulocytes. There was no eosinophilia or basophilia in either animal at any time and at no time were myelocytes found in the peripheral circulation. Nor was arrhythmia noted in the delivery of the cells to the blood stream. A more or less marked degree of neutrophilic leucocytosis was maintained through-

[3] Doan, C. A., and Zerfas, L. G., *J. Exp. Med.*, 1927, **46**, 511.

out the experiment without the development of a tolerance and subsequent lack of response to the later injections of the nucleinate. There was a relative and absolute lymphopenia of moderate grade with an occasional shower of monocytes. The erythrocyte and hemoglobin levels fell gradually in both rabbits from between 5,000,000 and 7,000,000 with 10 to 11.8 gm. of hemoglobin to between 3,000,000 and 4,000,000 and 6.6 to 7 gm. of hemoglobin. The color index at the beginning was 0.60 and at the end 0.66 in Rabbit 0-80 and 0.50 and 0.57 in Rabbit 0-81. That is, the anemia was due to a proportionate fall in hemoglobin and erythrocytes, indicative of mechanical limitation of erythropoietic foci in the bone marrow, such as that found in acute experimental tuberculosis.[4] Microscopic study of the marrow revealed an extensive myeloid hyperplasia probably sufficient to account for the red cell phenomenon.

At postmortem no gross pathologic changes were noted in any organs or tissues except spleen and kidneys. The spleen in each animal weighed 8.5 gm. but the capsules were not tense and on cut surface there was not the parenchymal bulging with profuse hemorrhage characteristic of an acute splenic tumor. The usual scattered follicles were observed but were widely separated by solid, grayish-pink, cellular tissue.

The left kidney in Rabbit 0-80 weighed 17.5 gm. and the right 18.5 gm., as compared with 18 gm. and 19 gm., respectively, for Rabbit 0-81. The capsule stripped readily and there were no hemorrhages. The usual striations of the cortex were partially obliterated.

The bone marrow of the long bones was pale grayish-pink, solid, not fatty, easily removed in intact pencils for microscopic study. No lymphoid hyperplasia was apparent either in the regional lymph nodes or in the Peyer's patches and diffuse lymphoid tissue of the gastro-intestinal tract.

The bone marrow on microscopic examination showed myeloid hyperplasia with marked diminution of fat. The most striking finding in the spleen was the presence of multiple small foci of myeloblasts and early myelocytes scattered throughout the parenchyma. Sections from the kidneys, more especially those from Rabbit 0-80, likewise showed multiple foci of myelocytes large and small, perivascular and inter-tubular, associated with glomerulo-tubular nephritis. Fig. 1 shows one of the larger periarterial foci in the kidney, Fig. 2 being a higher power of the central portion of the focus

[4] Doan, C. A., and Sabin, F. R., *J. Exp. Med.*, 1927, **46**, 315.

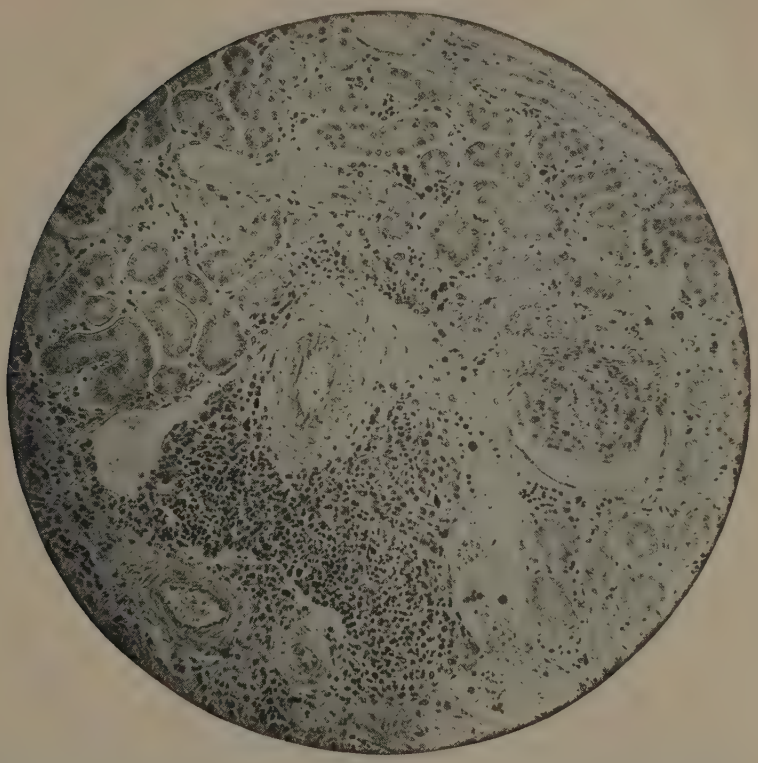

FIG. 1.
Extra-medullary focus of myelocytes in kidney parenchyma. Rabbit 0-80. × 150.

showing agranular myeloblasts and early myelocytes with varying numbers of specific granules. Neither basophilic nor eosinophilic myelocytes or erythroblasts were observed in any of these ectopic myelopoietic foci. In attributing these findings to the nucleinate molecule it may be remarked that this phenomenon has not been encountered previously either as a spontaneous or induced occurrence in an experience covering 13 years of intensive experimentation upon the cytological reactions of the rabbit.

A careful microscopic survey of multiple sections from the blocks of tissue representing the mesenteric, right and left inguinal and axillary, submaxillary and popliteal lymph nodes, liver, lungs, ovaries, heart muscle, adrenal and thyroid glands, failed to reveal myelopoietic activity or cellular infiltrations. The evidence of definite lymphoid hypoplasia is being investigated further.

Our experiments would seem to suggest 3 tentative conclusions:

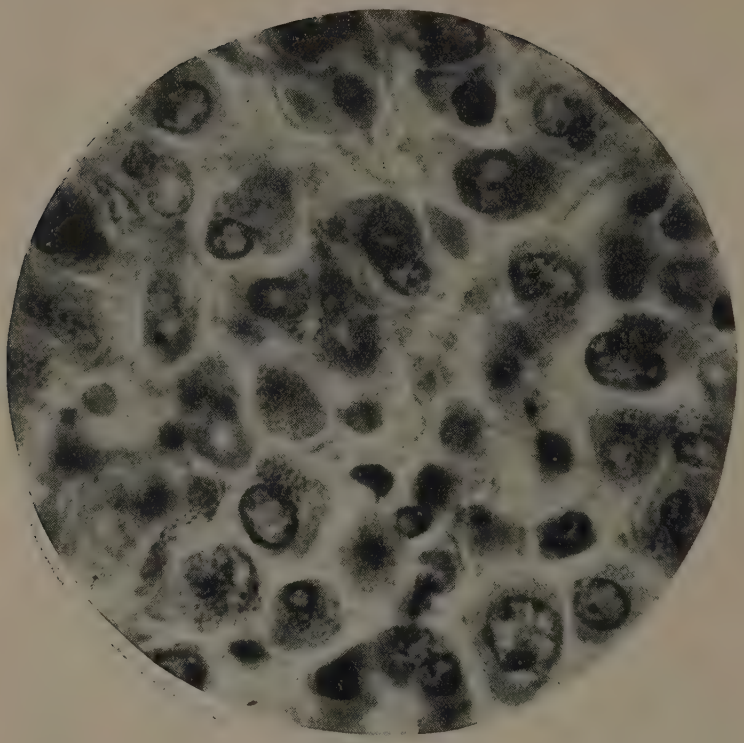

FIG. 2.
Individual myeloblasts and early myelocytes "A", "B", and "C" from the focus in Fig. 1. × 900.

(1) nucleic acid and its degradation products exert an immediate chemotactic effect on normal myeloid foci with a prompt, effective increase in the delivery of mature neutrophils to the circulation under a controlled rhythmic mechanism; (2) a short course of injections of the pentose nucleotides has resulted in the multiplication and maturation of myelocytes in normal marrow, without injury to the integrity of this or other organs of the body, and this reaction has been responsible for the neutrophilic leucocytosis observed in the blood stream; (3) repeated large intravenous injections of the nucleinate molecule have neither tended to exhaust the myelopoietic potentialities of marrow nor to cause a malignant hyperplasia of these elements in normal rabbits. Under the conditions of these experiments, a sufficiently specific and intensive stimulus to myeloid proliferation was present to occasion not only an extensive degree

of marrow hyperplasia but also to actually initiate autochthonous extramedullary myelopoiesis in kidneys and spleen.

6205

Effect of Light on Rats Receiving a Complete Diet.*

GEORGE O. BURR AND MARGARET SUTERMEISTER.

From the Department of Botany, University of Minnesota.

Conditions in our colony have been standardized as far as practicable. The diet is of the greatest simplicity and constancy.[1] The humidity is not controlled, but is recorded. The temperature is now maintained throughout the year at 26° ± 1°. The light is as uniform as natural light can be, coming through a window across the entire north wall of the colony. No direct sun enters the room.

Even with this uniformity of light from the north sky, it was thought that seasonal changes and location of cages might materially affect our experimental animals. At no time is there any ultraviolet light of antirachitic value in the room since the daylight is filtered through 2 thicknesses of window glass.

It is well known that light 2900-3100 A° in wave length has an effect on the metabolism of animals and is of very great importance if the animal is receiving a poor Ca:P ratio and lacks vitamin D in the diet. The recent work of Clausen[2] emphasizes the importance of a daily exposure of rats to infra-red radiation if the rats are rachitic. As little as 10 minutes a day of this light from a carbon arc with an energy value of only 0.122 cal/min/cm^2 was sufficient to markedly affect the rats.

The object of this experiment was to find out whether light materially affects the growth, food and water consumption, ovulation, lactation or bone analysis of rats maintained on our very simple but complete diets.

Albino rats (from our stock colony) were put in the experimental cages at weaning time (21 days after birth). They were kept in individual cages previously described.[1] The maintenance diet consists of pure casein 12.0%, sucrose 84.1%, salt mixture 3.9%,

* This work was made possible by a grant from the Committee on Radiation of the National Research Council.

[1] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345.

[2] Clausen, E. M. L., *J. Nutr.*, 1929, **2**, 125.

supplemented by the non-saponifiable matter from 70 mg. of cod liver oil and from 35 mg. of wheat germ oil fed on the daily yeast dose of 0.65 gm. This is a good diet when supplemented by 10 drops of corn oil.

It occurred to us that the rats might not respond to light treatment if the 10 drops of corn oil were omitted. This allows the skin to become scaly and dry. Therefore, 2 groups of animals have been maintained on this deficient diet.

Two dark rooms were built in the colony room and were ventilated with a blowing fan so that their temperature was almost exactly the same as that of the colony. These rooms were totally dark and were opened only when the colony room was dark. Very dim electric light was used while working with the animals. A total of 48 rats has been used.

Following Clausen's technique we irradiated half the rats in the dark rooms daily for 10 minutes with an Everready Sunshine Lamp,† (carbon arc, 13 amperes) at a distance of 1 meter.

After it was found that irradiation did not affect any of the groups materially the fat-starved rats were cured with 10 drops of corn oil so that reproduction could be studied. Therefore, the bone analyses are all on rats receiving fat.

The comparison of growth and food consumption of the different groups is given in Table I. There does not seem to be any

TABLE I.
Comparison of Growth and Ovulation by Groups Irradiated and Not Irradiated.

Group No.	Fat in Diet	Irradiation	Average wt. at age 84 days	Average wt. at age 175 days	Average daily food consumption during 6th month
	%		gm.	gm.	calories
67	2	Irradiated	145	189	36.5
69	2	Not irradiated	136	184	36.9
68	0	Irradiated	117	140	35.2
70	0	Not irradiated	127	144	36.0
71	0	Colony light	139	147	39.4

consistent difference between the animals in some light and those in total darkness. All groups consumed almost exactly the same number of food calories daily, except the rats in the main colony room (Group 71). The only explanation for this result is that the rats in the open room are disturbed more by workers and show more activity.

† We are indebted to the National Carbon Company, Cleveland, for the use of the lamp and carbons.

Records of ovulation and reproduction show no consistent difference between the irradiated and unirradiated animals. Ovulation rates and number of young born per litter may be said to be identical for comparative groups.

Bone analyses gave very similar results for all animals, whether irradiated or not (Table II). Chick and Roscoe's A/R[3] is high

TABLE II.
Average Bone Analyses for all Irradiated and Non-irradiated Rats.

Groups	Calcium (Dry wt. basis)	Calculated on Wet Weight Basis				
		Water	Fat	Ash	Organic Residue	A/R
	%	%	%	%	%	
67 and 68 (Irradiated)	21.6	26.2	5.2	44.2	24.3	1.82
69 and 70 (Not irradiated)	21.3	25.2	6.2	44.3	24.3	1.82

and identical for the 2 groups. The calcium agrees well with the standards of Korenchevsky.[4]

Conclusions. Rats reared on a highly purified and very simple diet do not suffer appreciably from living in total darkness. They show no consistent response to daily irradiation with an open carbon arc.

6206

Site of Hypersensitiveness of the Exaggerated Sinus Caroticus Reflex.

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From the Department of Medicine, University of Minnesota, Medical Service, General Hospital.

Before Hering's[1] discovery of the carotid sinus reflex, the slowing of the pulse which results from pressure over the upper area of the carotid artery, particularly on the right side of the neck, was interpreted as the direct effect of pressure upon the vagus nerve. Variations in the effect on heart rhythm were considered as indicative of the condition of the heart muscle and the Wenckebach[2] school

[3] Chick, H., and Roscoe, M. H., *Biochem. J.*, 1926, **20**, 137.

[4] Korenchevsky, V., Medical Research Council, Special Report No. 71 (1922).

[1] Hering, H. E., *Die Karotissinusreflex auf Herz und Gefasse.* Theodor Steinkopff, Dresden, 1927.

[2] Wenckebach, K. F., *Die Unregelmässige Herztatigkeit.* W. Engelman, Leipzig, 1914.

particularly attempted to use such differences in the prognostication of heart disease. Weil[3] agreed with Wenckebach that a prolonged cardiac arrest was due to a hypersensitiveness of the vagus endings in the myocardium consequent on pathological changes in the heart. Hering, having discovered that a reflex mechanism and not a direct stimulation is at play, became interested in the receptor organs of the afferent branch of the arc rather than in the end organs of the different efferent branches. He considered that a marked slowing of the pulse on pressure over the *sinus caroticus* was due to a hypersensitiveness of the afferent nerve endings induced by arterial sclerosis in this region. Consequently, Hering and his collaborators are inclined to use these same variations in heart rhythm after pressure over the carotid sinus as indicative of arterial sclerosis of the carotid artery. The experiments here reported were undertaken to determine whether the hypersensitivity in the exaggerated cardio-inhibitory response is in the afferent or efferent branches of the arc.

The demonstration by Hering[1] that pressure on the *sinus caroticus* elicits 2 independent effects, (1) cardio-inhibitory effect, (2) vaso-depressor effect, permitted the following approach to the problem. The afferent pathway for both reflexes is a common one by way of the sinus nerve through the glossophalangeal nerve to the central

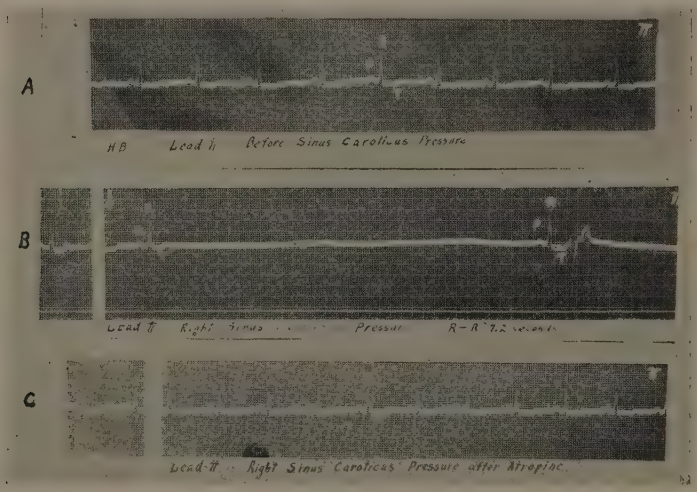

FIG. 1.
Electrocardiogram showing the effects of *sinus caroticus* pressure before and after atropine.

[3] Weil, A., *Deutsch. Arch. f. klin. Med.*, 1916, **119**, 39.

nervous system. There are 2 separate efferent pathways, by way of the vagus to the heart and by the sympathetic system to the blood vessels. The cardiac effect may be eliminated by atropine and the vascular reaction independently observed. Since the afferent endings are common to both reflexes, a marked cardio-inhibitory effect may be explained by hypersensitiveness of the afferent receptors if there is likewise a vascular reaction of a comparable degree. On the other hand, the combination of a marked cardiac inhibition and a mild effect on blood pressure may be interpreted as an indication of an increased activity of the efferent branch of the cardiac reflex, the vagus nerve.

The subject upon whom the following observations were made was a male, 70 years of age, with a history of mild anginal attacks. Over a period of several months his blood pressure varied between 130 mm. to 150 mm. systolic and 90 to 100 mm. diastolic. The response to pressure over the *sinus caroticus* was observed on numerous occasions with the string galvanometer. The reactions were constant, consisting of complete arrest of cardiac activity for periods varying from 7 to 9 seconds, with shorter periods on pressure over the left *sinus caroticus*. On March 3, 1932, the systolic pressure was 130 mm. and diastolic 90 mm. Pressure over the right *sinus caroticus* produced a cardiac arrest of 7.2 seconds (Fig. 1, B). Atropine sulphate, gm. 0.002, was administered subcutaneously. Twenty minutes after injection the blood pressure was 128 systolic and 90 diastolic. Pressure over the *sinus caroticus* on either side produced no effect on the heart rate. (Fig. 1, C.) On right *sinus caroticus* stimulation the arterial pressure dropped almost immediately to 110 mm. systolic and 90 mm. diastolic and rose within 2 minutes to 125 systolic and 90 diastolic. The effect of left *sinus caroticus* pressure was then observed. The blood pressure was 128 systolic and 90 diastolic, which dropped on pressure over the carotid sinus to 110 systolic and 90 diastolic, returning in 2 minutes to 130 systolic and 90 diastolic. From various reports this is to be considered a mild or moderate vascular response, since reductions in blood pressure of from 40 to 50 mm. with a return in from 10 to 15 minutes to the original level have been frequently observed. These observations indicate that the exaggerated cardio-inhibitory effect of *sinus caroticus* pressure is not the result of a hypersensitiveness of the afferent nerve or its endings.

6207

Factors Involved in the Regeneration of Hemoglobin.

H. L. KEIL AND V. E. NELSON.

From the Laboratories of Physiological Chemistry, Iowa State College, Ames, Ia.

All of the experiments were performed on rats. The animals were made anemic by feeding whole milk, collected in glass containers. The materials studied for hemoglobin regeneration were all tested for copper and used only when this element was absent. Hemoglobin was determined by the Newcomer method. The animals were bled by the tail.

There was no effect on hemoglobin when anemic rats were fed 1, 5, and 10 mg. Fe as $FeCl_3$ together with whole milk. We then studied the effect of tyrosine, tryptophane, glutamic acid, aspartic acid, and arginine on hemoglobin formation. The rats were fed on milk when 30 days old and after 4 weeks were given 100 mg. of amino acid plus 0.5 mg. of Fe as $FeCl_3$ daily. Regeneration of hemoglobin did not occur on any of the amino acids employed. The conflicting results in the literature on the potency of various elements in hemoglobin building may be due to different amounts being absorbed; to test this point we injected various salts intraperitoneally. The following elements were tested: As, Mn, V, Ge, Ni, Zn, Cr, Ti, Se, Hg, and Rb. Most of the metals were injected at levels from 0.05 mg. to 0.1 mg. daily. Arsenic was injected at 0.01 to 0.2 mg. daily. None of these elements caused regeneration of hemoglobin; in fact, the only element we have thus far found which has a positive effect is copper. Although we found that Fe as $FeCl_3$ when administered orally did not stimulate hemoglobin regeneration, we were anxious to ascertain if the same results would be obtained by intraperitoneal injection of the Fe. Three mg. of Fe as $FeCl_3$ injected every other day into anemic rats increased the hemoglobin from initial levels of 3.0 to 5.5 gm. to final values of 9.5 to 12.1 gm. per 100 cc. of blood. Injections of $FeCl_3$ are severe on the animal resulting in necrosis of the surrounding tissue at the point of injection. Intraperitoneal injection of aqueous ferric citrate or $FeCl_3$ dissolved in glycerine were found not to have the deleterious effect of aqueous $FeCl_3$ and to stimulate hemoglobin regeneration. The utilization of the iron under these conditions appears to be temporary. It does not mean a utilization of Fe to the exclusion of copper, but may denote a failure of absorption of iron in nutritional anemia. Five rats were made anemic by feeding milk. They

ranged in hemoglobin from 3.1 to 5.0 gm. per 100 cc. of blood. They were then given orally 0.05 mg. Cu as $CuSO_4$ daily and were injected every other day with 1 mg. of Fe as a suspension of $Fe(OH)_3$. The collodion dialysate of the $Fe(OH)_3$ showed no test for Fe by KCnS. After 11 weeks the final HHB values ranged from 13.2 to 16.3 gm. per 100 cc. of blood.

Detre[1] showed that hemorrhagic anemia responds to treatment with acids. This led us to a study of the effect of HCl on hemoglobin regeneration in nutritional anemia. The acid was prepared by decomposing $MgCl_2$ with H_2SO_4 and passing the HCl so formed into Cu free water through a drying train to dry the gas and prevent the carrying over mechanically of traces of Cu.

Four rats were made anemic with milk and had initial hemoglobin values of 6.5, 8.0, 7.4, and 8.2 gm. per 100 cc. of blood. They were then injected intraperitoneally with 1 cc. of 0.0333 N HCl every other day. After 5 weeks the hemoglobin values were: 6.8, 10.2, 8.7, and 11.0. A second lot of 5 rats were treated in the same way, except 1 cc. of 0.092 N HCl was used for the injection. These rats had initial hemoglobin values of 4.1, 4.0, 6.5, 4.3, and 7.2. The maximum values found in about 3 weeks were 8.7, 7.5, 8.9, 6.7, and 9.8 gm. per 100 cc. of blood. After this time the hemoglobin fell so that in 7 weeks the values were 6.0, 5.0, 8.6, 6.1, and 5.8. The acid injection was then discontinued and 0.01 mg. Cu as $CuSO_4$ was injected every other day. After 4 weeks the hemoglobin values were 12.0, 15.4, 16.2, 14.5, and 15.1. A third group of 4 animals were injected with 0.5 cc. of 0.092 N HCl every other day. The initial readings were: 6.1, 6.0, 5.7, and 5.7. The maximum hemoglobin levels reached in about 14 weeks were: 10.3, 11.5, 12.0, and 13.5. The last 2 animals died shortly after the final readings of HHB were made. At the end of 18 weeks, the first 2 animals had HHB values of 9.1 and 8.2. The first lot of rats on acid received $FeCl_3$ in the milk and the last 2 lots $Fe(OH)_3$, equivalent to 0.50 mg. of Fe daily. Acid apparently causes a temporary rise in hemoglobin, but later the level of hemoglobin falls.

[1] Detre, L., *Klin. Wochenschr.*, 1929, **8**, 1312.

6208

Immunity in Human Beings to the Pneumococcus.

ARTHUR F. COCA.

From the New York Hospital, the New York Postgraduate Hospital, and the Lederle Laboratories.

In the fall of 1930, I began a study of the products of several bacteria in the search for hitherto unknown exotoxins which could be identified as the pathogenic agents of the respective microorganisms. This paper is a summary of the findings with various culture filtrates of the pneumococcus.

In this work I have enjoyed the cooperation of Dr. Henry W. Straus and of Dr. Norman Plummer. We shall make a detailed report of the experiments, some of which are still in progress.

The existence of a pneumococcus toxin had been suspected and A. B. Wadsworth had recommended the injection of whole cultures rather than the collected bacterial bodies in the immunization of horses.

In view of the consistent failures to demonstrate the toxin in animal experiments it was decided to test the filtrates upon young and presumably susceptible children by subcutaneous injection; the criteria of toxic action were to be a local effect and a rise in temperature.

The experiments were begun with a freshly isolated culture of type II pneumococcus obtained from Dr. Plummer.

The first filtrates produced temperatures ranging from 101°F. to 104°F. in subcutaneous doses of 2.0 cc. to 4.0 cc. or of 0.5 cc. to 1.0 cc. of the filtrate concentrated by acetone precipitation according to the method of Wadsworth and Quigley.[1] There was tenderness and swelling at the site of injection.

The later experiments were carried out with an unconcentrated filtrate from a stock type I culture which produced temperatures of 101°F. and upward in a quantity of 0.5 cc.

Immunity to the fever-producing substance was established in some children after a single pyrogenic dose or 2, and in a series of 21 children of about 5 years of age, immunity was established in 14 within 2 weeks after a second injection.

The serum of the immunized children neutralized the pyrogenic substance in mixture or separately injected. For example, 1.0 cc. of the serum neutralized 4 minimum pyrogenic doses.

[1] Wadsworth, A. B., and Quigley, J. J., *J. Immunol.*, 1931, **20**, 459.

The serum of type I and type II pneumonia convalescents neutralized the pyrogenic substance. Normal children's serum in a quantity of 2.0 cc. did not neutralize a single minimum pyrogenic dose of the type I filtrate (0.6 cc.).

Not all toxic filtrates of pneumococcus cultures are antigenic. Of 5 children that received 3 injections of 2 or more minimum pyrogenic doses at weekly intervals of filtrates from ordinary phosphate broth cultures none was found immune upon the fourth injection one week later.

The study of the conditions favorable to the production of the antigenic toxin is continuing and the results will be published in the full report.

The toxin appears to be stable since none of the filtrates have shown any lessening of toxicity, one of them having been tested over a period of 4 months.

Skin Test. The undiluted type I filtrate injected intracutaneously in a quantity of 0.05 cc. produced within 20 hours, an area of erythema with swelling and tenderness of 1½ to 3½ inches in diameter in *all* of 14 children who were given at the same time 1.0 cc. of the filtrate subcutaneously. Twelve of these children developed temperatures ranging from 100.4°F. to 104.6°F. The other 2, both of whom had had pneumonia, showed temperatures between 98°F. and 100°F., which were considered within normal range.

These results seemed to indicate that the undiluted filtrate was too toxic for practical testing purposes since the 2 immune children exhibited no difference in skin reactivity to the filtrate as compared with the 12 susceptible children.

One week later, 11 of these 12 were tested again with the undiluted filtrate and 5 of them responded with greatly diminished reactions (¾ inch or less). One refused to be tested.

A second subcutaneous injection (of toxoid—described below) was given and one week later 6 that showed no diminished reaction were tested with 0.1 cc. of the filtrate diluted 1-10. At this test 4 showed reactions of ⅞ inch to 1¼ inch diameter which were considered positive; the result in the other 2 was negative—slight redness of ¼ inch diameter or less.

The further results with the skin test, which was carried out with the 1-10 dilution of the type I pneumococcus filtrate, are summarized in the table.

The results of this limited number of tests seem to indicate that, as is the case in immunity to diphtheria, the number of immune

TABLE I.

Results of Cutaneous Tests with 1:10 Dilution of Filtrate of Type I Pneumococcus.

	Positive	Negative
Medical interns; laboratory and office staff; nurses	31*	18†
Ward patients	16	31
Pneumonia convalescents	1‡	26§

* One of these had had one attack of pneumonia and one had had 2 attacks.

† Five of these are known to have had pneumonia.

‡ Dr. Plummer regards the diagnosis of lobar pneumonia in this case as doubtful; an "unclassified" pneumococcus was found in the sputum.

§ Of these, 7 cases were of type I, 3 of type II, 2 of type III, 3 of type IV, 3 of type V, 1 of type VIII, 1 of type XX with Friedlander, and 7 of "unknown" strains.

adults is greater among the class of people represented by ward patients than among a professional class.

Pneumococcus toxoid was prepared as usual by adding formalin to the undiluted type I filtrate in a concentration of 0.3% of formaldehyde and incubating for 4 weeks at 37°C.

Ten children received 2 injections of 1.0 cc. at one week interval and 2 weeks later were tested with the 1-10 toxic filtrate. At this test 3 showed positive reactions (⅞ inch in diameter) the others, negative ones (¼ inch or less). The first toxoid injection produced a temperature of 103°F. in one child, 101°F. in a second and no rise in the others.